AUTHOR INDEX

e, D. 929
ır, F. M. 1727
ıs, H. K. 480, 1474, 1525
ss, J. S. 685
El Hamid, A. 1094
El-Salam, M. H. 611
l-Halim, S. M. 1908
l-Latif, M. F. 1023-1024
l-Malek, E. 1450
l-Rahman, H. A. 269-270
-Alla, M. 1314
-El-Bakey, A. 1314
nathy, A. R. 1992
all, B. 1105
Elnaga, I. G. 610, 1676
ı-Heilah, A. N. 105
l-Khier, F. 180, 1094
l-Khier, F. A. 1095, 1127
m, D. D. 245
mov, A. V. 1354
mson, D. 1523
lea, O. 121
f, G. 150
ne González, R. 484
nicki, F. 1033
nov, E. V. 1324, 1382
kaveg, J. E. 1760
n, E. 713
iyun, A. A. 10, 246
a, V. A. 124, 131, 1035
O. O. 281
cyan, E. K. 773
bio, M. 514
ekov, N. M. 1200
wal, R. 128
wal, R. K. 526
wal, S. C. 1780
rwal, P. K. 1714
rwal, V. 517
wal, O. P. 1830
culture Canada 1852
ad, M. A. 64
ad, N. 274
ad Said, S. 1771
ed, A. 936
ed, A. A. 1127
ed, A. A. H. 180
ed, M. S. H. 1645, 1932
ed, S. M. 470, 1285
J. S. 1139
R. 39
T. 664
ra, K. 978
ıg, H. 802
ane, T. 324
ar, M. 1662
ienko, V. K. 1386
ni, F. 811
wadi, A. 912
zawi, A. F. 1854
akkak, Z. S. 1256, 1645, 32
amando, M. M. 1021
wad, I. M. 169, 181
aliky, S. K. 1645
baidi, Z. S. 1021
ıud, H. M. 1854
ıwaf, S. D. 1683
ogair, A. M. 550
bhaily, I. A. 1522
, R. O. 1629
ru, M. E. Herrera 1675
ıa Alfonso, H. M. 266
sandrov, M. 1485
ieva, V. 1155
K. A. 1695
ander, M. 304, 762, 1958
ıso, H. M. Aldana 266
M. F. 269-270
S. R. 1645, 1932
M. 376, 1848
, G. J. 1004
Y. O. 938, 1546
nan, J. E. 1974
Allen, C. 1653
Allen, J. C. 600
Allotey, J. 984, 1289
Allsopp, D. 5, 1407
Allsopp, P. G. 1540
Alonge, D. O. 165
Alston, A. S. 666-667
Alterthum, F. 97
Altmayer, B. 100, 992
Aly, H. El-S. 70
Aly, H. Y. 72
Amador, J. A. 1958
Amaral Filho, B. F. 707
Amarowicz, R. 626, 1674
Amemiya, J. 170
Amer, M. I. 710
Ammar, I. M. A. 1582
Amodio, R. 1096, 1575
Amrani, N. 504
Anand, S. K. 1020
Anderegg, B. N. 1555
Anders, C. 2031
Anderson, D. G. 1789
Anderson, J. E. 686
Anderson, M. E. 1066
Anderson, P. H. 1064
Anderson, T. M. 775, 810
Anderson, W. B. 2031
Anderson, W. E. 247
Andotra, P. S. 505
Andrews, S. 1444
Andrews, W. H. 405
Andreyuk, E. I. 1220-1224, 1228-1229
Anees, I. 1916
Angel, S. 1060
Anisimova, L. A. 1345
Anjali Roy 1742
Anjana Singh 1631
Annis, P. C. 743
Annual Report, Cyprus Agricultural Research Institute 1036, 1243

Antonovskaya, N. S. 1221, 1224
Aoki, S. 1255
Aoust, J. Y. d' 1156
Apeland, J. 1049
Appleton, N. 407
Aquarone, E. 808, 2038
Arbogast, R. T. 1586, 1925
Archer, D. 148
Archer, D. B. 317
Arinze, A. E. 531
Armstrong, J. W. 117, 1623, 1638
Arnason, J. T. 738
Arora, D. S. 1331
Arora, K. L. 1081
Arora, M. L. 1953-1954
Arora, S. K. 1648
Arthaud, J. F. 163
Arya, A. 128
Arzamastsev, A. A. 1380
Asaduzzaman, M. 1732
Asakura, J. 1973
Ascanio, E. 913
Aschenbach, J. M. 1449
Asiedu, S. K. 1054
Asperger, H. 594, 618, 623
Asseldonk, W. van 1082
Aston, D. 1401
Atanasov, Kh. 1271
Atkey, P. T. 384
Atkin, P. 450
Atkinson, J. C. 989
Atmawinata, O. 983
Atri, D. C. 1008
Attia, F. I. 279, 719, 1882
Attia, M. F. 72
Aturaliya, D. S. 848
Australian Centre for International Agricultural Research 382, 1495
Autar, D. K. 88
Avakyan, Z. A. 814
Ávila, F. A. de 1475
Avilés Pacheco, R. 66
Ayadi, A. 298
Ayala, A. Castillo 568-569
Ayaz, M. 550
Aydın, M. 471
Ayertey, J. N. 1521
Azawi, A. F. Al- 1854
Azevedo Rodrigues, Z. 59
Aziz, F. M. 1854
Babenko, Yu. S. 804
Babitskaya, V. G. 1319, 1363
Babos, K. 663
Babsky, N. E. 876
Badawi, A. 1765
Badawy, A. S. 1683
Badr-Eldin, S. M. 1364
Baechler, R. H. 1807
Baeva, S. Y. 1313
Bagley, R. W. 1919
Bagnara, C. 344
Bahareth, T. O. 550
Bailey, J. S. 15, 172
Bailey, P. 6, 75, 263, 285-288
Baines, E. F. 1194
Bajpai, R. 2048
Baker, G. H. 257
Baker, J. E. 1858, 1895
Baker, R. 557, 995
Balagopal, C. 334
Balasubramanian, M. 727
Balasubramanian, T. 449
Balasundaran, M. 647
Baldwin, R. C. 1165
Ballabene, N. C. 425
Ballinger, W. E. 1029
Balloni, W. 808, 2038
Balock, J. W. 1597
Baltes, W. 1061
Bánáty, P. 1713
Bancroft, M. N. 519
Bandler, R. 989
Banerjee, M. 370
Banerjee, T. C. 924, 1512
Banks, H. J. 743, 1262, 1501, 1886
Banks, J. G. 1121
Banwart, G. J. 402
Bapat, D. R. 948
Bär, M. 1769
Barak, A. V. 276
Barakat, F. M. 72
Baranowski, J. D. 154
Barbosa, M. de F. S. 1361, 2019
Barchia, I. 1478
Bardtke, D. 1358
Barik, S. 358
Bariska, M. 203, 1747
Baritelle, J. L. 519
Barker, P. S. 290, 754
Barlik, Z. 1129
Barnard, R. J. 89
Barnes, C. P. 1945-1946
Barnoud, F. 636
Barry, T. L. 1450
Bars, J. le 907
Barth, E. F. 1953-1954
Bartha, I. 1712
Bartl, V. 860
Bartov, I. 918
Bartsch, H. 844
Barve, S. S. 397
Bărzoi, D. 184
Bassalik-Chabielska, L. 1123
Bassam, B. A. 806
Bassi, M. 1823
Bassiony, T. A. El 180, 1127
Bassiony, T. El 1094-1095
Bata, A. 1488
Batchelor, T. A. 1607-1608
Bateman, P. L. G. 824, 1281
Batish, V. K. 177, 564, 1099
Battistotti, B. 577, 1717
Bauerfeind, S. 1304
Bauman, L. F. 462
Baur, F. J. 1399
Bautista G., A. 994
Bayer, E. A. 1736
Bazak, M. 113
Bean, G. 880
Beard, J. T. 1591
Beasley, J. N. 65
Beaver, R. A. 264
Bebee, C. N. 76, 86, 111
Bech-Andersen, J. 1227
Bechinski, E. 1508
Becker, H. 1684
Beckers, H. J. 572, 594, 1434, 1664
Bedard, D. L. 1329
Bedere, C. 879
Bedlan, G. 133
Beeman, R. W. 1865, 1880, 1885
Beerens, H. 970, 1393, 1433
Beesley, J. 1401
Beever, D. J. 1051
Beg, M. A. A. 326
Begg, N. 1661
Behr, L. 1609
Beker, M. 329
Belaich, J. P. 344
Belkanova, N. P. 814
Bell, C. H. 271, 736, 741, 1886
Bell, D. K. 36
Bell, M. F. 1066
Bell, R. G. 28
Bellés, X. 1257
Bellows, T. S., Jr. 268
Beltrones, J. S. 1887
Belyaev, S. S. 798
Ben-Arie, R. 113
Ben-Efraim, A. 475, 1579
Ben-Yehoshua, S. 104, 527
Benezet, H. J. 1590, 1934
Bengston, M. 71, 750
Benjamin, M. M. 2058
Benjinali, B. 298
Benkerroum, N. 1085
Benkhemmar, O. 445
Benner, R. 791
Bennett, E. O. 1213
Bennett, G. A. 878, 955
Benschoter, C. A. 1016
Bentley, W. J. 975
Berchieri Junior, A. 1475
Berg, S. 39
Bergdoll, M. S. 1449
Berger, H. 146
Berger, H. K. 293
Berger, J. A. 1836
Berger, L. R. 1836
Beritić, T. 488
Berman, P. M. 1653
Bernáth, I. 1682
Berry, R. W. 660
Bertona, A. M. 594
Bertrand, J. C. 1959
Betina, V. 55
Betts, W. D. 228
Bettucci, L. 646
Beuchat, L. R. 92, 477, 1075, 1656
Beumer, R. R. 1101
Beveridge, T. J. 1348
Bezanson, G. S. 847
Bezborodov, A. M. 817
Bhalla, S. K. 1689
Bhargava, S. N. 119
Bhatia, S. K. 717
Bhookul, N. 722

Bhullar, J. S. 1637
Biely, P. 303, 789, 2019
Bigalke, D. 174, 189, 194, 584, 1087, 1115
Bijker, P. G. H. 1665
Bilai, T. I. 1237, 1239, 1315
Bilous, D. 346
Binns, T. J. 1935
Birewar, B. R. 925, 927, 953
Biró, G. 1124
Bishop, J. R. 1707
Bishop, P. L. 307
Biswas, A. C. 1732
Biswas, S. R. 2070
Bitran, E. A. 60-61, 289, 935
Blachly, T. R. 1966
Blackall, L. L. 368
Blackwell, B. A. 414
Blanchette, R. A. 200, 209, 1756, 1761
Bland, M. A. 1719
Blaney, B. J. 446
Blankenship, L. 160
Blankenship, L. C. 248, 549, 553
Blease, K. S. 1719
Bleou, C. Brou 1056
Blocher, J. C. 406
Blomquist, G. 1832
Błoszyk, E. 730-731, 734
Blow, D. P. 678
Board, R. G. 1121
Boatright, R. E. 679
Bobbi, M. E. Cordal de 1679-1680
Boczek, J. 1566
Boddy, L. 1173
Bodie, E. A. 810, 1379
Bodnaryk, R. P. 290
Boer, E. de 489, 1431, 1440
Boisen, F. 1677
Boisvert, R. N. 2044
Boland, J. A. Vázquez 1131
Bolen, P. L. 1332
Bolland, L. 1391
Bolt, S. J. 167
Bolton, F. J. 3
Bompeix, G. 1602, 1606
Bonants-Van Laarhoven, T. M. G. 1594
Bonassi, I. A. 1098
Bond, E. J. 110, 749, 1922
Bonhomme, A. 1160
Boone, T. 1994
Booth, R. H. 137
Bopp, L. H. 1329
Borah, B. 69
Borden, J. H. 715, 1180, 1497, 1516, 1878-1879
Borges, A. Chaer 1361
Borisova, T. I. 804
Boronin, A. M. 797, 1345
Bos, A. P. 1447
Bosman, M. T. M. 242
Bosshard, H. H. 203
Bossuyt, R. 178, 185
Botha, W. C. 1137
Bottalico, A. 899, 1143
Bottazzi, V. 577
Bouchot, F. 352
Bourgeois, C. 1105
Bourgeois, C. M. 1105
Boutin, B. K. 1663
Bouvier-Fourcade, I. 445
Bowen, M. R. 1934
Bowley, C. R. 736
Bowry, S. K. 1937
Boyd, S. A. 1355, 2029
Bracewell, A. J. 549, 553
Brackett, R. E. 1656
Braid, G. H. 637
Bramlage, W. J. 999-1000
Brandl, D. G. 1929
Brandl, E. 618, 623
Braquehaye, C. 1120
Bravery, A. F. 683, 701, 1822, 1834
Bravova, G. B. 1387
Breeze, A. S. 1820
Bremser, J. F. 686
Brennan, M. J. 1329
Brennan, P. W. 956
Brewer, I. N. 1568
Bridges, J. T. 1919
Brink, W. J. van den 1395
Briozzo, J. 873
Britton, A. 909
Britz, T. J. 765, 2051
Brocklehurst, T. F. 1157
Brooks, F. W. 1787
Brooks, R. F. 1017
Brou Bleou, C. 1056
Brower, G. R. 2008
Brower, J. H. 1926-1927
Brown, A. E. 630
Brown, E. J. 2024
Brown, G. E. 521
Brown, J. A., Jr. 1819
Brown, R. E. 497
Browne, F. G. 656, 1248
Browne, K. M. 1641
Bruce, W. A. 1856
Bruinenberg, P. M. 2068
Brulla, W. J. 358
Brumley, W. C. 1450
Bruna V., A. 1650
Bry, R. E. 679, 1816
Bryan, F. L. 70, 538
Bryant, C. W. 1980
Bryant, M. P. 358
Bubyrenko, T. A. 2043
Buchanan, J. R. 40, 1013
Buchanan, R. L. 388, 858, 1069
Buchanan, S. A. 286, 740
Bucharová, M. 1699
Buchelos, C. T. 985
Büchmann, N. B. 1062
Buck, W. B. 1722
Buckle, A. E. 435
Buckle, A. P. 1910
Bueschkens, D. H. 1078
Bühler, N. B. 1092
Buhr, K. L. 969
Bulatao-Jayme, J. 424
Bulbul, O. 1506
Buller, H. 542
Bullerman, L. B. 33, 1028
Buras, N. 1306
Burdaspal, P. A. 1467
Burditt, A. K., Jr. 1597
Burg, W. R. 1527
Burge, W. D. 760
Burkholder, W. E. 276, 282, 1865, 1874, 1888
Burlingame, G. A. 1293
Burns, R. B. 919, 1080
Burzo, I. 501
Busta, F. F. 27, 406, 545, 1839
Butani, D. K. 94
Butcher, J. A. 1739
Byrne, A. 1804
Cail, R. S. 1816
Cairney, J. W. G. 206
Calderon, C. 26
Calderon, M. 1506, 1856
Campano, S. G. 252
Campbell, D. J. V. 771
Campbell, D. S. 493
Campos, T. B. 60-61, 289, 935
Canada, Agriculture Canada 1852
Canadian Pest Management Society 825
Candlish, A. A. G. 38
Cannon, C. 1240
Cannon, P. F. 1844
Cantone, F. A. 462
Capoor, A. K. 2049-2050
Cappellini, R. A. 1034, 1055, 1639
Carey, J. K. 1948
Carini, E. 571
Carini, S. 594, 1705
Carisano, A. 616
Carlson, M. K. 1824
Carman, A. S. 1725
Carpenter, J. A. 549, 553, 867
Carter, F. L. 219
Carter, S. W. 1786
Carteron, B. 879
Cartledge, M. F. 1130
Carvalho Neto, C. de 1909
Casas, E. de las 1430
Casolari, A. 173
Castaño, Z. J. 942
Castegnaro, M. 844, 887
Castillo Ayala, A. 568-569
Castro, A. F. P. de 408
Caswell, G. H. 82-83
Cavanaugh, J. A. 1262
Celano, G. 187
Centre for International Agricultural Research, Australia 382
Centre for Overseas Pest Research, United Kingdom 822
Centro Internacional de Agricultura Tropical 960, 1390
Ceponis, M. J. 1034, 1055, 1639
Cerniglia, C. E. 336, 355
Cervantes, L. A. 506-507
Chaer Borges, A. 1361
Chakrabarti, N. 1632
Chakraborty, B. K. 1695
Chakravarty, A. 370
Chalmers, W. T. 1381
Chalutz, E. 121, 513, 1027
Chambers, J. 1890
Champ, B. R. 1503
Chamy M., R. 362
Chan, H. W. S. 444, 1064
Chan, K.-Y. 147
Chanchareonsook, J. 1311
Chand, H. 1535
Chanda, S. 2000
Chander, H. 177, 470, 564, 1099, 1285
Chandla, V. K. 1041
Chandler, R. E. 579
Chandra, R. 927, 953
Chandra, S. 490
Chandrakala, T. 1916
Chandrakantha, J. 1570
Chandramohan, D. 24, 540
Chandrasekaran, M. 24, 540
Chang, F. C. 17
Chang, H. L. 893
Chang, W. M. 1139
Changjaroen, P. 936
Chapman, A. H. 230
Chapman, D. A. 12
Chartron, S. 851
Chaudry, S. S. 1695
Chauhan, K. S. 512
Chauhan, S. 122
Chawanapong, M. 1539
Chełkowski, J. 472, 899, 1074
Chen, G. C. 249
Chen, Q. Z. 1509
Cherdyntseva, T. A. 628-629
Cherif, R. 1549
Chernysheva, N. K. 675, 1201, 1790
Chessa, I. 1019
Chet, I. 106, 351
Chew, V. 528, 979
Chhinnan, M. S. 477
Chiao, J. S. 1962
Chib, H. S. 505
Chiba, S. 60-61, 289, 935
Chiddle, T. C. 1189
Chiesa, S. C. 2039
Childs, D. P. 1591
Chile, S. K. 127
Chiou, R. Y.-Y. 92
Chirife, J. 873
Chittenden, C. M. 1210, 1800
Chiusa, P. 577
Chmielewski, W. 712, 724
Chmielowski, J. 2004, 2062
Cho, C. M. 1513
Chohan, J. S. 1640
Chong, C. E. 547
Chong, K. F. 1206
Choo, H. Y. 214-215
Chopin, A. 594
Chopra, G. 274, 1911
Christensen, S. G. 1429
Christoffersen, A. B. 1677
Chronas, G. 401
Chu, F. S. 34, 423, 1466
Chu, T. J. 2064
Chuan, L. 468, 1920
Chuang, Y.-T. 308
Chubb, R. 578
Chudoba, J. 1975
Chung, N. K. 2063
Chung, Y. W. 655
Chuvil'skaya, N. A. 1386
Chynoweth, D. P. 1983
Ciegler, A. 41, 1529
Ciesielska, Z. 1251
Ciobanu, M. 1872
Citrograph 1436, 1624
Claflin, J. K. 1557
Clark, A. G. 1078
Clark, C. S. 1957
Claus, G. 335
Clement, B. A. 1471
Clevström, G. 415
Cline, L. D. 855, 1550
Coates, D. 648-650
Cochat, N. 1111
Cock, J. 424
Cockcroft, R. 1187
Coetzer, J. J. 745, 1272
Coffelt, J. A. 976
Cogan, P. M. 1889
Cogburn, R. R. 1888
Coggins, C. R. 2078
Cohen, H. 432
Coiffier, O. 1706
Coker, R. D. 843
Cole, R. J. 979
Coleman, W. 238
Coleman, W. W. 238
Collard, P. 20
Collazos E., O. 994
Colley, R. H. 1197-1198
Colli, I. A. G. 408
Collins-Thompson, D. L. 2
Cominazzini, C. 594
Compare, G. dal 1681
Conconi, J. Ramos-Elorduy e 1887
Conde, A. R. 1999
Condemine, G. 698
Conn, W. M. 1972
Conner, D. E. 1656
Conrad, J. R. 1983
Conradie, W. E. 1184, 118 1799, 1802
Constant, J.-L. 879
Conway de Macario, E. 36
Conway, W. S. 114
Cooke, B. C. 562, 1671
Cookson, L. J. 1750
Coombs, C. W. 1496
Coombs, J. 2085
Cooper, D. G. 366, 2060
Corcoran, E. 774
Corcoran, R. J. 118
Cordal de Bobbi, M. E. 16 1680
Corden, M. E. 677, 1203, 1850
Cordray, J. C. 159
Côte, L. M. 1722
Couey, H. M. 117, 1623
Coughlan, M. P. 339
Coulter, J. 435
Counter, D. E. 3
Cousin, M. A. 21, 588
Coutin, R. 687
Cowan, B. W. 2055
Cox, J. 936
Cox, J. M. 607
Cox, N. A. 15, 172
Craven, S. E. 248
Crawford, D. L. 1733
Crawford, R. 705
Crawford, R. L. 251, 2024
Credland, P. F. 1564, 1857 1862
Croan, S. 1385
Crosby, N. T. 882
Crosby, T. K. 1728
Cross, D. J. 1792
Cross, M. 63

ckshank, J. G. 395
z, M. L. 902
z, M. R. de M. 97
jesi, A. J. 1804
va Barajas, L. de la 1887
nington, A. M. 1904
uerella, J. 1031
an, P. M. T. 652
in, M. E. 691
er, C. S. 546
comp, L. K. 923
c, Z. 1083
orek, S. 826
bour, A. 1765
ngsubha, W. 971
ley, S. 1323
er, N. S. El 156
lem, A. M. 1722
Z. R. 657, **1767**

ty, R. H. 166
Monte, G. 837
, B. E. 1334
eet-Singh, K. 225
ien, P. M. M. 1047
oglou, A. P. 48, 493, 934
ce, S. J. 292
do, T. R. 1408
iel, D. 1849
iewski, W. M. 734
ish Pest Infestation Laboratory 2071
ud, S. M. 1448
oust, J. Y. 403, 1156, 1489
ust, R. A. 1565
C. N. 505
gan, R. A. 16
, R. K. 981
K. 1532
S. K. 2001
en, A. 1142
ilva, E. J. 758
, E. S. 1649
yuk, N. M. 681
e, J. M. 1696
idson, J. I., Jr. 979
idson, P. M. 1217, 1950
ies, J. 1370
ies, R. 1076-1077
ies, R. A. H. 71
ies, R. M. 143
is, B. 769, 1312
is, P. G. 1987
is, R. 1500, 1549, 1566
, S. W. 461
B. C. 671-672
Conconi, J. Ramos-Elorduy 387
a Cueva Barajas, L. 1887
as Casas, E. 1430
Lima, C. P. F. 747
Moraes Rêgo, A. F. 59
Oliveira, M. Lindete 59
Souza Leão Veiga, A. F. 59
con, J. W. 343
k, T. 107, 1394
evere, J. M. 1448
ker, G. 337
leire, M. 809
e, E. B. 981
root, R. C. 1196, 1745, 1773
el, K. E. 160
el, R. H. 29
azos, E. D. 511, 1009
quis, P. J. 557
š, F. 488
gou-van Asch, E. 1664
gou-Van Asch, E. H. M. 4
ittle, D. W. 1402
, T. R. 219
nas, C. 850, 870
nas, C. L. 857
nain, A. L. 352, 1409
eş, S. 502
mark, Nordic Wood Preservation Council 1186
nis, C. 103
ton, H. 530
Deppe, H. J. 226
Dergileva, A. N. 1299
D'Errico, M. M. 1096, 1575
Desai, B. B. 495
Deschamps, A. M. 332
Deschatelets, L. 790
Desher, D. P. 2032
Desmarchelier, J. M. 71, 742, 751, 1917
Detroy, R. W. 374
Detzler, W. 903
Deutsche Veterinärmedizinische Gesellschaft 2079
Devi, G. R. 1452
DeVries, J. W. 893
Devriese, L. A. 1071
Dexter, J. E. 956
Dhawan, S. 1830
Diab, M. M. 977
Dias, R. 1266
Díaz S., J. 914
Dick, K. M. 1564, 1857
Dicke, B. 1711
Dickens, J. A. 172
Dickens, J. W. 844, 979
Dickie, N. 1662
Diepolder, H. 560
Diermayr, P. 244
Dieter, H. O. 674
Dietz, M. G. 232
Dijke, J. F. van 1004
Dijken, J. P. van 2068
Dikshit, A. 1947
Dillman, L. L. 29
Dimitrova, M. 350
Diprossimo, V. 1450
Dirol, D. 1795, 1913
Dix, N. J. 206
Dixon, B. 1956, 2010, 2013
Dixon, J. B. P. 1272
Dizer, H. 1964
Djenepo 1056
Dobie, P. 838
Doboszyńska, B. 1129
Dobriyal, P. B. 1814
Dockerty, T. R. 1070
Dodd, G. D. 824, 1258
Dodgson, K. S. 799, 1352
Doelle, H. W. 1962, 1997
Doharey, R. B. 961, 965
Dohroo, N. P. 1593
Dolan, A. 524
Dolfing, H. 314
Dolgikh, L. M. 804
Domínguez Rodríguez, L. 1131
Dominik, J. 273, 659, 1179, 1205, 1770
Domoto, M. 897
Don-Pedro, K. N. 1242
Donahaye, E. 1266, 1506
Donèche, B. 96
Donefer, E. 322
Donovan, J. 1970
Doores, S. 160
Đorđevic, J. 816
Dorozhkin, N. A. 1313
Dörtbudak, N. 471
Dott, W. 1294
Douglas, P. E. 461
Downey, G. A. 934
Downing, F. S. 1259
Downing, S. F. 824
Dowson, C. G. 1173
Doyle, M. P. 160
Drake, M. 999
Dreu, R. de 1594
Drift, C. van der 767
Driguez, H. 349
Drożdż, B. 730-731, 734
Duarte, M. H. 1694
Dube, H. C. 1849
Dube, S. 1949
Dublish, P. K. 1941
Duck, D. 847
Dudchenko, L. G. 1238
Duek, L. 1306
Dufrenne, J. 534
Duguet, J. S. 1056, 1569
Dul'tseva, N. M. 783
Dumontet, S. 318
Duncan, S. H. 367
Dunkel, F. V. 468, 1920
Dunn, R. 2069
Duraković, S. 487-488
Duraković, Z. 487-488
Duran, A. P. 89, 148
During, M. 4
Dürrigl, A. 895
Dutt, N. 723
Dutta, S. 1742
Dutton, M. F. 875
Dvali, G. N. 49
Dwivedi, D. K. 119
Dwivedi, P. 919, 1080
Dwyer, D. F. 2029
Dyte, C. E. 272
Dzhusupova, D. V. 1345
Eagon, R. G. 1945-1946
Eamus, D. 1847
Eaton, R. A. 1401, 1759
Eckert, J. W. 126, 519, 866
Edgington, L. V. 525
Edney, K. L. 112
Edwards, R. A. 166
Edyvean, R. G. J. 694
Effland, M. J. 200
Efstratiou, M. 401
Egli, T. 2021
Egmond, H. P. van 51, 844, 1686
Egorova, L. A. 783
Egorova, Z. E. 438
Ehlers, J. G. 548
Ehrentreich, W. 826
Ehrlich, K. 41, 1529
Eichhorn, K. W. 100
Eighmy, T. T. 307, 1965
Eisa, A. A. 1582
Eischen, G. W. 1982
Eisenberg, E. 1060
Eklund, M. W. 541
El-Bahrawy, A. 47
El-Bassiony, T. 1094-1095
El-Bassiony, T. A. 180, 1127
El-Deep, S. H. 1059, 1063
El-Diwany, A. I. 2018
El-Fouly, M. Z. 168
El-Gayar, F. H. 1268
El-Gazzar, F. E. 706, 1468
El-Goorani, M. A. 142
El-Haidari, H. S. 1854
El-Hamady, A. M. 90
El-Helaly, M. S. 1268
El-Huseiny, T. M. 168
El-Kady, I. A. 883
El-Kasheir, H. M. 142
El-Kazzaz, M. K. 142
El-Mahdy, R. M. 1059, 1063
El-Marrakchi, A. 1085
El-Mongy, T. M. 168
El-Nabawi, A. 1582
El-Nabawy, S. M. 90
El-Nimr, A. A. 1364
El-Nockrashy, S. 580, 1692-1693
El-Rab, H. G. 1095
El-Rayes, E. 348
El-Said Aly, H. 70
El-Samkary, M. A. 1059, 1063
El-Samragy, Y. A. 581, 1690-1691
El-Shaieb, M. K. 456
El-Sherbeeny, M. R. 70, 538
El-Shibiny, S. 583
El Sonbaty, M. R. 501
El-Tobshy, Z. M. 1023-1024
El-Wahab, F. A. 90
Elder, W. B. 1554
Eller, K. I. 49, 617
Elliott, H. J. 1402
Elliott, T. J. 384
Elnaga, I. G. Abo 610, 1676
Elphinstone, J. G. 1044
Elsmore, R. 1818
Emel'yanova, L. A. 1790
Emovon, O. 1724
Engel, G. 589
Engelhardt, G. 896
Enkiri, N. K. 760
Ennis, B. M. 1374
Enríquez, A. 338
Entis, P. 863-864, 1432
Epino, P. B. 80
Erdos, G. W. 766
Eriksson, K. E. 636
Ermush, N. A. 1208
Ertola, R. J. 1375
Escartín, E. Fernández 568-569
Esentepe, M. 87
Eshaque, A. K. M. 1732
Eslyn, W. E. 200, 633, 1752
Ethiraj, S. 101
Etter, L. 1712
Eulitz, E. G. 291
European Homologation Committee for Wood Preservatives 1183
Evans, D. E. 288, 1504, 1557
Evans, N. J. 737
Evanson, M. L. 1449
Ewais, S. M. 611
Ewings, K. N. 1103
Fadelmula, A. 1543
Fahim, M. M. 72
Fahmi Saddik, M. 70
Failla, L. J. 1530
Fairbairn, D. J. 1232
Faison, B. D. 1734
Fan, T. S. L. 34
Fandino, G. E. 158
Fane, A. G. 1557
Fang, F. 1833
Fannin, K. F. 1983
Farag, F. 1522
Faragalla, A. A. 1275, 1765
Farber, J. M. 403
Farid, M. A. 2018
Farkas, G. 1122
Farkaš, V. 303
Farrell, R. L. 1734
Fauchille, S. 325
Faustini, D. L. 1923
Favarin, V. 11
Favennec, P. 1142
Fay, H. 1270
Federici, F. 253, 2034
Fedorak, P. M. 2031
Fejér-Balogh, É. 1682, 1713
Feldmesser, J. 2084
Felip, G. de 1425
Ferguson, J. F. 2058
Fermor, T. R. 321, 384
Fernandes de Moraes Rêgo, A. 59
Fernandez-Coll, F. 555
Fernández Escartín, E. 568-569
Fernández, G. Suárez 1131
Fernández Garayzábal, J. F. 1131
Fernando de Souza Leão Veiga, A. 59
Fernando, T. 880
Ferrari, A. 1823
Ferraz, C. A. M. 808, 2038
Ferreira, M. S. 996
Ferri, E. Rodriguez 1131
Feschter, A. 373
Fiechter, A. 250
Filimonova, T. V. 1215
Filip, Z. 772, 1964
Finch, G. R. 2006
Fincham, J. E. 54
Findlay, W. P. K. 202, 1401
Finland, Technical Research Centre of Finland 827
Finne, G. 150-151
Finnegan, B. 1508
Fiore, P. 1096, 1575
Fischer, J. R. 1322
Fisher, K. T. 1253
Fitzner, M. S. 969
Flaherty, B. R. 1550
Flanagan, J. F. 1106
Flannigan, B. 461, 1456
Fleet, G. H. 171, 396, 558, 869
Flegg, P. B. 384
Fleurat Lessard, F. 1499, 1556
Floetenmeyer, M. D. 361, 363

Florenzano, G. 808, 2038
Floyd, J. B. 867
Foda, Y. H. 1059, 1063
Fogel, M. M. 1328
Fogel, S. 1328
Foglia, T. A. 1106
Foglietti, M. J. 1160
Folchi, A. 1604
Fonty, G. 1160
Forbes, G. I. 604
Forbito, P. R. 876
Ford, J. E. 1719
Forest Research Institute, New Zealand 1775
Forfang, J. C. 1116
Forsberg, C. W. 345, 793
Fortlage, R. J. 40, 1013, 1646
Fougerousse, M. 1401
Fougerousse, M. E. 1193
Fourie, J. F. 1615-1616
Fournaud, J. 163
Fox, T. L. 545, 1720
Foxall-VanAken, S. 1819
France, Société Française de Microbiologie 1393
Francioso, E. 187
Francis, O. J., Jr. 1725
Frank, M. 831
Franke, V. 559
Frankland, C. A. 1528
Franklin, J. D. 1617
Franzmann, B. A. 1540
Frecker, T. 279
Freeman, J. P. 336, 355
Freer, S. N. 374
Freitas, J. P. G. V. de 391
Freke, C. D. 562
Fremy, J. M. 1146
Frevel, H. J. 589
Friedlander, A. 1928
Friend, D. W. 1464
Friesen, M. 844
Frolov, I. P. 998
Fu, Y. K. 474
Fuchs, Y. 121
Fugel, S. 529
Fujii, Y. 1748
Fujikawa, S. 1301
Fujinuma, K. 901
Fukatani, Y. 1973
Fukumori, F. 788
Fukushima, H. 543, 1669
Fuller, W. H. 1307
Fuzeau, B. 1556
Fyler, J. 1547
Gabler, E. C. 2044
Gaby, L. I. 673
Gajdůšek, S. 601, 1699
Gale, J. 738
Galil, N. 1960
Galli, A. 1855
Gallo, F. 1827
Gamal, N. F. 1320
Gamal, R. F. 1320
Gambassi, D. M. 97
Ganciu, M. 502
Gandy, D. G. 384
Ganesalingam, V. K. 1869
Gangwar, S. K. 1280
Gannon, J. E. 1213
Garayzábal, J. F. Fernández 1131
Garces, M. C. 1887
Garcha, H. S. 1480
García-Aguirre, G. 1526
Garcia, D. L. 1638
Garcia-Hernandez, F. 1359-1360
Gardner, P. D. 519
Garg, A. K. 728
Garg, S. R. 1090
Garibaldi, A. 509
Gariepy, Y. 1050, 1054
Gashe, B. A. 929
Gasser, J. K. R. 828
Gathercole, P. S. 426
Gatley, S. 407
Gaudemer, F. 445
Gaudin, C. 344
Gaudy, A. F., Jr. 1966, 2028
Gaur, V. K. 1458
Gaya, P. 192, 622
Gayar, F. H. El- 1268
Gaze, R. H. 384
Gbodi, T. A. 938, 1546
Gecan, J. S. 989
Geeson, J. D. 1641
Geiger, J. P. 1758
Geiger, R. M. 1213
Gélinas, P. 299
George, J. 1401
Gerats, I. A. G. M. 1594
German Federal Republic, Deutsche Veterinärmedizinische Gesellschaft 2079
German Federal Republic, Deutsches Institut für Normung eV 670
German Federal Republic, Institut für Bautechnik 1204
Gerson, U. 725
Gersonde, M. 226, 1912
Gesche, E. 561
Ghaleb, H. M. 1136
Ghaly, T. F. 1554
Ghazi, I. M. 1314
Gherghi, A. 502
Ghodeker, D. R. 595
Ghoneim, N. H. 591
Ghose, T. K. 2015
Ghosh, A. 819
Ghosh, B. K. 819
Ghosh, J. 958
Ghosh, S. N. 1559
Gibbins, L. N. 345, 793
Gibbs, P. A. 46
Gibson, A. H. 1340, 1344
Gibson, D. G. 1745
Giginyak, Yu. G. 1384
Gijsegem, F. van 1236
Gijzen, H. J. 2007, 2014
Gilbert, J. 414, 434, 458, 615, 891, 972, 993, 1147
Gilbert, W. 1955
Gilbertson, R. L. 1406, 1760
Gill, T. 770
Gillespie, W. J. 2057
Gillis, W. T. 1130
Gilmour, A. 1107, 1708-1709
Gimeno, A. 427
Gindrat, D. 1046
Ginn, R. E. 238, 1720
Ginns, J. H. 651
Giraffa, G. 1705
Girgis, E. S. 583
Girish, G. K. 941
Glaeser, H. 619, 1093
Glatz, B. A. 361, 363
Glaze, L. E. 989
Glenn, J. K. 197
Glenn, M. 819
Glick, B. R. 821
Gnanaharan, R. 647, 1729, 1766
Goater, B. 2080
Gobran, F. H. 1560
Gogov, I. 871
Gohsh, S. P. 129
Goicoechea Mayo, A. 1687
Gold, M. H. 197, 376, 1848
Goldner, S. B. 278
Goldstein, R. M. 762
Gołębiowska, Z. 711
Goliński, P. 472, 1074
Golob, P. 936, 1892
Golovacheva, R. S. 1225
Golovchenko, N. P. 1386
Golovko, A. I. 1313
Golovleva, L. A. 1345, 1356
Gomes, C. 1694
Gomes, M. de F. F. F. 1073
Gomyoda, M. 1669
Gonzaga, E. 577
González, A. 208
González Valenzuela, M. 1571-1572, 1859
Goodchild, D. J. 1339
Goodell, B. S. 223
Gool, A. van 779
Gorevoi, M. R. 1201
Gorlatov, S. N. 798
Gorris, L. 767
Gosselé, F. 1635
Goswami, N. P. 520
Goto, T. 412, 982
Gottschal, J. C. 2035
Goud, H. D. 357
Gouhar, K. A. 710
Goulet, J. 299
Govers, H. 802
Goyal, G. K. 566
Grabarczyk, H. 730-731, 734
Grabarkiewicz-Szczęsna, J. 1074
Grace, J. K. 1182
Gradidge, J. M. G. 1389
Graham, A. F. 1838
Graham, R. D. 223, 1796, 1801, 1808
Granovsky, T. A. 258
Grant, C. 683, 701, 1822
Grant-Mackie, E. 1607
Grant, W. D. 321
Grappin, R. 1142
Graves, R. R. 686
Gray, D. R. 1180
Grayston, S. J. 1346
Grbić-Galić, D. 792, 2020
Greaves, H. 1750
Green, J. L. 1471
Greenhalgh, M. 387
Greenhalgh, R. 414
Greening, H. G. 748, 1498, 1867
Greenwood, B. F. de 1744
Greer, G. G. 1068
Gregory, K. F. 331, 340
Grichanova, G. S. 1201
Grierson, W. 1012
Griffin, D. B. 548
Griffioen, A. 314
Griffiths, E. T. 1353
Griffiths, M. W. 563, 576, 1104, 1721
Grigorov, N. I. 783
Grigoryan, K. M. 617
Grigoryev, E. F. 804
Grinda, M. 639
Grobler, I. 544
Groff, K. A. 2059
Gross, C. 1955
Grosser, D. 840
Grover, N. K. 1091
Guan, L. H. 1730
Guaraldi, F. 1641
Guaycurús, T. V. 391
Gueguen, M. 1235
Guiffre, V. 286, 740
Guimarães, W. Vieira 1361
Guindani, C. M. A. 996
Guirguis, M. W. 710
Gulevskaya, S. A. 801
Gullino, M. L. 509
Gumargalieva, K. Z. 1215
Gupta, A. K. 122, 1648
Gupta, A. P. 739
Gupta, B. R. 505
Gupta, G. P. 926
Gupta, J. S. 84-85
Gupta, O. P. 512
Gupta, P. C. 974, 1284
Gupta, P. K. 56, 85
Gura, I. S. 681
Gurprasad, N. P. 52
Gutter, Y. 120
Gutteridge, C. S. 130
Gutzmer, D. I. 220
Guy, E. J. 1106
Guzhova, E. P. 1387
Haast, J. de 765, 2051
Haberl, M. L. 1329
Haddrell, R. L. 714
Hagagg, L. F. 90
Hagler, W. M., Jr. 1148
Hagstrum, D. W. 968-969, 1551, 1873
Hahn, G. 594, 620
Hahn-Hägerdal, B. 1367
Haidari, H. S. El- 1854
Haigler, J. H. 158
Haines, C. P. 838, 1246
Hakkak, Z. S. Al- 1256, 1932
Hald, B. 436, 1062
Halderson, J. 1508
Hale, M. D. 1759
Hales, S. G. 1352-1353
Halid, H. 1267
Halim, S. M. Abdel- 190
Hall, E. R. 1976
Hallenberg, N. 2082
Halliday, D. 272
Halsall, D. M. 1339-1340, 1344
Halstead, D. G. H. 1257
Halter, N. 1376
Hamami, M. S. 1771
Hamami, S. M. 1771
Hambleton, P. 407
Hamdan, I. Y. 319
Hamelin, M. 1706
Hamer, G. 319
Hamid, A. Abd-El 1094
Hamilton, R. M. G. 1464
Hammond, E. G. 361, 36
Hammond, J. B. W. 384
Hamoudi, R. F. 81
Hamparian, V. V. 301
Han, M. 1785
Han, S. H. 1828
Handley, A. 676
Hang, Y. D. 2044
Hangst, E. 619
Hanlin, R. T. 207, 483, 1
Hanna, M. O. 548
Hansen, K. 565
Hantschke, B. 1809
Haralampu, S. G. 1995
Hardjo, S. 983
Hardjoutomo, S. 183
Harper, S. H. T. 342
Harris, D. L. 1265
Harrison, M. A. 867
Hart, L. P. 47, 417
Hartford, W. H. 1198
Hartin, K. E. 1464
Hartley, D. 1889
Hartman, T. G. 433
Hartog, B. J. 1440
Harun, J. 1287
Haruta, M. 179
Harvey, J. 1107
Hasegawa, A. 881, 888, 8
1461, 1513-1514
Hasegawa, Y. 515
Hassan, A. A. 581
Hassan, N. R. 1881
Hassell, M. P. 268
Hasuo, T. 1296, 1300-13
Hauschild, A. H. W. 32,
Havelaar, A. H. 4
Havlová, J. 1083
Hawa, S. G. 171
Hawaiian Sugar Planters' Association 1824
Hawksworth, D. L. 375
Haworth, S. R. 482
Hayashi, N. 68
Hayashi, T. 1255
Hayashida, S. 1845
Haydak, M. H. 1872
Hayes, A. W. 844
Hayward, A. C. 368
Heale, J. B. 143
Heather, N. W. 1861
Heatherbell, D. A. 1636
Hedén, C. G. 1962, 1997
Hedley, M. E. 1791
Heerden, F. R. Van 54
Heeschen, W. 574-575, 5
594, 598
Heflich, R. H. 355
Hefnawy, S. A. 611
Hegarty, B. M. 652
Heidelbaugh, N. D. 1471
Heinrich, G. 697
Heinrichs, E. A. 1534
Heinritz, B. 1997
Heinz, D. J. 1824
Heisick, J. 861
Heitkamp, M. A. 336
Helaly, M. S. El- 1268

erblom, W. C. A. 1484
nann, E. 697
ns, C. W. 1934
ng, G. G. 1796, 1801
phill, D. D., Jr. 1383
fricks, L. H. 1547
s, Y. 475, 1579
c, L. L. 1334
ningsson, B. 1163
nlich, W. 865
issat, B. 349
schel, K.-D. 1052
ert, B. N. 1825
nandez, M. P. 160
e, D. H. C. 110
era, A. 66
era Alberu, M. E. 1675
ain, A. 610, 1676
eltine, C. W. 463
leis, M. 293
nanski, M. T. 440
ish, J. 1661
itt, J. H. 1661
ard, C. M. 166
s, K. B. 1106
, G. A. 1045
shida, S. 764
gins, T. E. 2032
nland, H. A. 855
nley, T. L. 198, 631, 633,
5, 1169, 1175, 1746
uchi, T. 347, 841, 1159
D. W. 885
E. C. 1724, 1818
R. 230
R. A. 1585
R. J. 1557
W. E. 874
er, J. C. 668
s, R. E. 1985
heimer, R. 32
on, B. L. 2009
on, H. W. 364
i, Y. 764
ta, T. 93
tsuka, Y. 634
koto, H. 416, 418, 901
K. K. 695
king, A. D. 385
kney, L. J. 588
ges, R. J. 530, 838, 1267,
860
son, R. E. 791
g, M. G. 1051
ink, A. J. 1968
o, H. 935
aday, C. E. 979
derna, E. 235-236
e, B. D. 271, 736
ó, J. 2072
oway, B. A. 196
oway, G. J. 473, 928,
574
n, D. G. 141
mberg, K. 240
mes, D. S. 2005
mes, M. 615
singer, V. H. 1106
t, D. 1740
ub, M. 734
z, G. 1615-1616
z-Zentralblatt 688
g, J. A. 1318
g, J. S. 372
g, L. T. 1162
d, M. A. 1230
f, J. van 1071
lwerf, J. D. 1703
per, S. W. 359
ver-Litty, H. 207
kins, T. L. 1880
ák, E. 1473
ber, E. 1502, 1543, 1568
ikoshi, K. 788
itsu, H. 313
sley, S. D. 3
vath, D. J. 1310
kins, C. M. 364
sono, A. 1372
ssain, M. M. 1654
chkiss, M. W. 1617
Houwaard, F. 779
Howard, D. C. 1876
Howell, H. N., Jr. 216
Howell, M. 441
Howitt, C. 263
Hrudey, S. E. 2006
Hrušovský, J. 906
Hsieh, D. P. H. 412
Hsieh, K. M. 693
Hsieh, Y. L. 1817
Hu, T. 474
Huang, Z. Y. 657
Huck, P. M. 2031
Hudson, H. J. 1757
Hudson, W. R. 1067
Huffman, B. B. 1934
Huffman, D. L. 159
Hughes, J. H. 301
Hugouvieux-Cotte-Pattat, N. 698
Huh, C. S. 586
Huhtanen, C. N. 157, 1069
Humphrey, T. J. 395
Husain, A. 1947
Husain, S. S. 64
Hussain, A. F. 1256
Hussein, A. M. 1646
Hussong, D. 760
Hutchins, A. S. 1270
Hutchins, J. E. 1148
Hutchinson, D. N. 3
Hüttermann, A. 351
Huyhn, N. van 809
Huynh, C. H. 849
Huynh, S. 849
Huynh, V. B. 251, 705
Hylin, J. W. 1623
Iannotti, E. L. 1322
Iba, Y. 515
Ibe, A. 901
Iben, C. 623
Ibrahim, A. A. 499
Ibrahim, G. F. 396, 869
Ibrahim, M. A. 1275
Ichinoe, M. 978, 1561
Idaka, E. 313
Idise, O. E. 98
Ihlal, M. 298
Ikediobi, C. O. 938, 1546
Ikemoto, H. 1295
Ikonomova, A. 350
Il'chenko, A. P. 801
Illian, M. 912
Imamura, Y. 201, 1748
Imanaka, Y. 902
Imbroglini, G. 1606
Imura, O. 73
Incze, K. 1394
India, Central Food Technological Research Institute 380
India, Timber Development Association 2077
Ingram, J. M. 1348
Inoi, T. 324
Insect Identification Sheet, Agriculture Canada 259-261
International Potato Center, Peru 1042
Iordăchescu, C. 529
Irvine, R. L. 1953
Ishihara, M. 700
Ishii, K. 881, 1561
Ishikawa, H. 641
Ishitani, T. 93
Isigidi, B. K. 1071
Islas-Murguia, L. 1359
Ismailov, N. M. 1351
Israĕlidēs, K. I. 608
Israilides, C. J. 608
Ito, M. 1296
Itoh, Y. 978
Ivanova, I. I. 784
Ivanova, N. 1777
Ivbijaro, M. F. 1519
Ivoilov, V. S. 1325-1326
Iwakiri, A. 170
Iwamura, N. 902, 980
Iwanski, M. L. 2064
Iwatsu, T. 255
Iwuagwu, Y. O. U. 1595
Izat, A. L. 150
Izuagbe, Y. S. 98, 1595
Jackai, L. E. N. 1565
Jackman, R. 437
Jacks, T. J. 464
Jacob, G. 1695
Jacobson, M. 1918
Jacobson, R. 920
Jacquet, J. 1235
Jadhav, L. D. 1651
Jae, J. W. 655
Jäger, A. 1385
Jagow, J. A. 89
Jagtar Singh 1990
Jain, J. C. 1191
Jaipal, S. 284
Jairam, B. T. 1097
Jaksch, P. 573
Jaksevac, J. R. 816
James, A. P. 2003
Jamrichová, S. 1110
Jandaik, C. L. 1341
Jang, H. S. 890, 1513
Janossy, G. 1133
Jans, J. A. 594
Janse, J. 1030
Jansen, A. 669, 1799
Jansen, J. T. 1446
Janssen, F. W. 489
Janssen, P. 779
Janštová, B. 1699
Jarry, M. 79
Jarvis, G. 594
Javaheri, M. 356
Jay, E. 720, 1585
Jayachandran, T. 1113-1114
Jayanata, Y. 2048
Jayaraj, S. 727
Jayaram, M. 744
Jayaraman, K. S. 690, 1991
Jeffries, P. 1829
Jeffries, T. W. 365
Jelley, W. C. N. 3
Jenkins, D. 1977-1978
Jenkins, R. K. 1069
Jenneman, G. E. 356
Jennings, D. H. 1847
Jennrich, H. 539
Jeong, J. C. 1318
Jerger, D. E. 1983
Jeris, J. S. 1984
Jervis, D. I. 594
Jesenská, Z. 997
Jessop, E. G. 3
Jiang, C. Y. 1828
Jičínská, E. 1083
Jiménez-Cuesta, M. 1031
Jiménez Pérez, S. 1687
Joblin, K. N. 367
Jodral Villarejo, M. 190
Johnson, B. R. 220, 249, 1843
Johnson, C. 1329
Johnson, D. B. 353
Johnson, D. D. 548
Johnson, E. A. 352
Johnson, K. M. 1977-1978
Johnson, L. M. 1292
Johnson, Z. B. 65
Johnston, E. M. 1600
Johnston, J. H. 1161
Joia, B. S. 1548
Jonas, K. 1490
Jones, B. D. 843
Jones, D. 256
Jones, F. G. W. 836
Jones, M. G. 836
Jones, R. D. 1938
Jones, T. 1
Jong, S. C. 492
Jongejan, A. 2061
Joo, Y. S. 1135
Jooste, P. J. 1137
Jordaan, E. 544
Jordano Salinas, R. 190
Jorgensen, M. A. 1671
Joseph, G. 600
Joshi, I. J. 1657
Joshi, V. K. 592
Journal of the South African Veterinary Association 35
Judy, L. A. 1116
Jung, G. T. 372
Juven, B. J. 99, 1060
Kabeel, M. T. 142
Kabondo, P. 879
Kader, A. A. 1013
Kafel, S. 1718
Kagimura, T. 315
Kahlon, S. S. 592, 1091, 2001
Kahrer, A. 145
Kale, P. N. 1040
Kalogridou-Basileiadou, D. 606, 609, 1154
Kalogridou-Vasiliadou, D. 606, 609
Kalogridou-Vassiliadou, D. 1154
Kalra, J. S. 498
Kalra, K. K. 1814
Kalra, M. K. 1846
Kalter, R. J. 2044
Kamalam, P. T. 818
Kamaya, Y. 1159
Kamel, A. H. 57
Kamimura, H. 410
Kampelmacher, E. H. 572, 1101, 1434
Kandler, O. 182
Kang, A. S. 444
Kanhere, S. R. 956
Kanjilal, S. C. 925, 927, 953
Kansal, B. D. 1990
Kapich, A. N. 1313, 1363
Kaplan, A. M. 1291, 1330
Kaplan, D. L. 1330
Kaplan, H. J. 523
Käppeli, O. 1376
Käpylä, M. 241
Karahadian, C. 29
Karaioannoglou, P. 625
Karaoulanis, G. 109
Karasevich, Yu. N. 780-781, 1325-1326
Karavaiko, G. I. 795, 814, 1225, 1324, 1382
Karcılıoğlu, A. 87
Karppanen, E. 39
Karpukhin, V. H. 1354
Karunamoorthy, G. 1481
Karunasagar, I. 1660, 1660
Kasashkina, T. P. 1347
Kasatkina, V. I. 1349
Kashyap, S. R. 666
Kasteren, J. Van 311
Kasumova, S. A. 990
Kataoka, M. 664
Katiyar, P. N. 963
Katiyar, R. N. 961, 965
Kato, S. 399
Katona, F. 1124
Kaur, P. 1583
Kautter, D. A. 31
Kawada, K. 522
Kawamura, K. 902
Kawashima, K. 1255
Kaya, S. 50, 915
Kays, S. J. 1038
Keck, H. S. 1979
Kędzia, B. 235-236
Keefer, R. F. 1310
Keenan, J. D. 1982
Keever, D. W. 1586
Kehagias, C. H. 608
Kehagias, H. H. 608
Keinath, T. M. 1979
Kelley, R. L. 1241
Kelley, T. R. 885
Kelly, M. J. 824
Kemp, D. L. 2052
Kendrick, B. 846
Kennedy, M. S. 1974
Kenny, J. W. 2025
Kent, R. J. 1298
Kerbabaeva, A. A. 998
Kerbel, E. L. 1010
Kerner, G. 1769
Kerner-Gang, W. 639, 1176
Kerner-Gang, W. von 1912
Kessler, W. 621
Ketchum, L. H., Jr. 1953
Keybets, M. J. H. 534
Khakhria, R. 847

Khalaf, S. H. 1672
Khan, B. A. 64
Khan, B. L. 567, 1701
Khan, N. R. 64
Khan, T. N. 1249
Khanbegyan, R. A. 911
Khara, H. S. 517
Kharatyan, A. M. 911
Khare, B. P. 963
Khier, F. A. Aboul 1094-1095, 1127
Khier, F. Aboul 180, 1094
Khmeleva, N. N. 1384
Kibata, G. N. 1520
Kibler, M. L. 247
Kientz, C. E. 429
Kiermeier, F. 884
Kierstan, M. 774
Kierstan, M. P. J. 774
Kiewnick, L. 537
Kikuchi, Y. 901
Kim, B. R. 2059
Kim, D. H. 1318
Kim, H. U. 586
Kim, J. M. 1318
Kim, J. W. 585
Kim, M. K. 1318
Kine, B. B. 102
King, A. D., Jr. 43-44
King, E. F. 312
King, R. R. 414
Kingsland, G. C. 1524
Kinner, N. E. 1965
Kinsman, D. M. 252
Kiran, U. 1480
Kirby, L. K. 65
Kirilenko, O. A. 438
Kirk, T. K. 1385, 1734, 1841
Kirsch, I. 889
Kishan Singh 2049-2050
Kiss, I. 1394
Kistemaker, A. C. 1728
Kitada, T. 399
Kitagawa, H. 522
Kitamura, K. 2065-2066
Kitani, M. 932
Kłapcińska, B. 2004, 2062
Klečka, G. M. 2023
Kleijntjens, R. 803
Klemm, K. 1366
Klich, M. 1529
Klostermeyer, H. 244
Klyushnikova, T. M. 1347, 1349
Knapp, J. L. 1017
Knapp, R. M. 356
Knauft, D. A. 969
Knowles, M. E. 1147
Knutson, D. 1270
Kny, U. 658
Kobayashi, J. 1561
Koburger, J. A. 16-17, 868
Kobzeva, H. Ya. 817
Kocheleff, P. 879
Kochetkov, V. V. 797
Kodikara, C. P. 848
Koehler, P. E. 92, 477
Koh-Knox, C. 462
Köhler, W. 1769
Kohno, N. 902
Koidis, P. 625
Kolmodin-Hedman, B. 1832
Komae, H. 68
Komarova, G. N. 801
Kombila-M., E. 1317
Komorowska, Z. 2033
Kompiang, I. P. 1479
Konečný, S. 1704
Konieczna, E. 2041
Konishi, K. 664
Konno, K. 764
Konopka, A. 2011
Kontová, M. 1715
Kopteva, A. E. 1228-1229
Kopteva, Zh. P. 1220, 1222-1223, 1228-1229
Koreshkov, N. G. 1324, 1382
Korlath, J. A. 1116
Korostyleva, R. N. 681
Korotkiya, G. Ya. 1208
Korsholm, E. 1678
Korzhenevich, V. I. 1357
Kosaric, N. 2046
Kosikowski, F. V. 188
Koske, R. E. 704
Kossen, N. W. F. 1368
Kostova, N. 1155
Kotimaa, M. 1170
Kotlarek, J. 1129
Kotula, A. W. 252
Kotzedidou-Rouka, P. T. 1438
Kovács, F. 1487-1488
Koval', A. A. 1349
Kovalenko, V. A. 1299
Kozak, J. J. 1716
Kozloski, R. P. 916
Kozlova, I. A. 1221, 1224
Kozuka, H. 886
Krahmer, R. L. 223, 1745
Krajewski, K. J. 640
Krall, S. 62
Kramer, K. J. 1547, 1865, 1880
Kratochvil, B. 2031
Krause, H. P. 910
Krauss, A. 638
Krauter, M. 2038
Krauzova, V. I. 801
Kriaa, H. 163
Krishna, S. S. 1274, 1853
Krishnaiah, J. 1627
Krishnamurthi, P. S. 1113-1114
Krishnamurthy, T. 430
Krishnamurthy, T. S. 744
Krishnarajah, S. R. 1869
Krishnaswamy, S. 1097
Kristofóri, K. 1713
Kritsepi-Konstantinou, M. 1084
Krogh, P. 436, 844
Królikowska, K. 1123
Kroll, R. G. 1102, 1688
Kropp, B. R. 210, 1850
Krug, M. 2026-2027
Kruk, M. 1566
Krumbein, W. E. 796
Krumme, M. L. 2029
Krupka, M. 1292
Kuan, S. S. 1725
Kubena, L. F. 1471
Kubeskaya, S. L. 1349
Kuchenrither, R. D. 1967, 1969
Kudo, T. 788
Kudryk, L. 290
Kueh, C. S. W. 147
Kuenen, J. G. 768, 803
Kugelman, J. J. 1984
Kühne, H. 658
Kulesh, V. F. 1384
Kulesza, J. 199
Kulkarni, K. A. 1545
Kulkarni, P. R. 397
Kulmanov, M. E. 892
Kulshreshtha, U. V. 1158
Kulshrestha, D. C. 567, 1701
Kumar, A. 1264
Kumar, G. 1086, 1088-1089
Kumar, O. 360
Kumar, R. 984
Kumar, S. 1779-1780, 1814
Kumar, V. 360
Kumaraswami, T. 922, 1250
Kumari, C. K. 1465
Kunath, P. 182
Kuntsmann, G. 1449
Kurane, R. 1327
Kurata, H. 416, 418, 829
Kurek, C. 613, 1125
Kurokawa, Y. 324
Kuter, G. A. 1968
Kutzner, H. J. 335
Kuwahara, M. 315
Kvasnikov, E. I. 990, 1347, 1349
Kwaga, J. K. P. 10, 246
Kwak, I. S. 1139
Kwaśna, H. 472
Kwolek, W. F. 878, 955
Labosky, P. 1278
Labosky, P., Jr. 1287
Labots, H. 1666
Lacey, J. 1476-1477
Lachance, M. A. 794
Lacy, G. H. 1653
Lacy, K. M. de 28
Ladaniya, M. S. 1637
Lafont, P. 844
Lagarde, E. A. de 873
LaGrange, W. S. 1128
Lai, B. 128
Lake, D. E. 686
Lakhani, G. K. 943
Lakshmanaperumalsamy, P. 24, 540
Lal, H. 1625
Lal, J. 1701
Lal, P. 1511
Lalić, L. M. 487-488
Lam, P. F. 116, 499, 1018, 1618, 1655
Lamb, R. J. 481
Lambert, J. D. H. 738
Lambrev, E. 1741
Lamed, R. 1736
Lan, Y. Q. 547
Lancette, G. A. 1435, 1445
Lang, J. H. 679, 1816
Langeland, W. E. 1981
Langeveld, L. P. M. 1703
Langner, K. J. 885
Langstrof, H. 1711
Lanier, J. M. 89, 594, 1435
Laohaviranit, L. 696
Lapointe, M. 432
Lara, F. M. 944
LaRocco, K. A. 491
Las Casas, E. de 1430
Lastick, S. M. 2002
Lata, P. 1423
Latif, M. A. 671-672
Latta, R. K. 2019
Latter, P. M. 1164
Lau, O. L. 1001
Laubscher, A. 544
Lauret, T. H. 1773
Law, B. A. 1232
Lawlor, T. E. 482
Lay, L. L. 413
Layrisse, M. E. 152
Le Patourel, G. N. J. 1558
Leadbeater, B. S. C. 2083
Leão Veiga, A. F. de S. 59
Leatham, G. F. 1343, 1841
Leclercq, A. 1749
Lecos, C. 1710
Ledeboer, A. M. 1447
Ledford, R. A. 1070
Lee, A. W. C. 665
Lee, B. H. 164, 1316-1317
Lee, C. K. 214
Lee, C. M. 1134
Lee, C. U. 300
Lee, F. L. 492
Lee, H. 2003, 2019
Lee, H. K. 776
Lee, J. O. 1139
Lee, K. B. 372
Lee, K. R. 1318
Lee, L. 1529
Lee, L. S. 464, 482
Lee, M. H. 655
Lee, S. F. 345, 793
Lee, S. K. 116
Lee, T. K. 1318
Lee, U. S. 890, 1461, 1513-1514
Lee, W. C. 1135
Leesch, J. 1549
Leesch, J. G. 1856
Leibee, G. L. 1053
Leisola, M. 250
Leisola, M. S. A. 373
Leistner, L. 420
Lem, N. W. 821
Lemarinier, S. 1235
Lensing, H. H. 757
Lenz, M. 1767
Lenz, P. 1457
Leong, P. C. 116
Leonova, V. E. 1354
Lepom, P. 431, 905, 1486
Lepschy, J. 896
Leschber, R. 1964
Lesniewski, R. S. 686
Lessard, F. F. 466, 1499, 3
Lester, J. N. 2030
Leuschner, A. P. 1321, 19
Leusden, F. M. van 572, 1
Levi, M. P. 642
Levinson, A. R. 296, 985, 1510
Levinson, H. Z. 296, 1510
Levitin, N. 1333
Levy, A. 1706
Levy, J. F. 1782
Levy, P. F. 1321
Lewis, G. 603
L'Heureux, L. 162
Li, C. H. 67
Li, G. X. 1247
Li, S. W. 1466
Lider, O. 725
Lidster, P. D. 1600
Liebert, C. A. 1230
Liese, W. 632, 1774
Liewen, M. B. 1150
Ligan, C. 1519
Lightner, G. W. 1034, 105 1639
Lilienthal, W. 560
Lilja, A. 1171
Lill, R. E. 535
Lillard, H. S. 1670
Lilly, T., Jr. 31
Lim-Sylianco, C. Y. 1902
Lima, C. P. F. de 747
Lin, H. H. 21
Lin, T. 67
Lindete de Oliveira, M. 59
Lindfors, E. 39
Lindgren, B. S. 715
Lindner, P. 1060
Lindsay, D. G. 439
Lindsay, R. C. 29
Line, M. A. 637, 1914
Linko, P. 2045
Linse, E. S. 1623
Linsell, A. 844
Lion, L. W. 693
Lior, H. 847
Lipschitz, N. 2078
Lira Pimentel, M. 59
Lisker, N. 475, 1579
Líšková, M. 303
Litchfield, J. H. 2036
Litopoulou-Tzanetakē, E.
Litopoulou-Tzanetaki, E. 1112
Littel, K. J. 491, 556
Little, C. R. 1025, 1621
Liu, M. T. 417
Liu, X. J. 1015
Ljunggren, H. 415
Lloyd, H. L. 1528
Lobos, J. H. 2005
Lodi, R. 594
Loehr, R. C. 1961
Loginova, L. G. 1387
Logvinovskiĭ, V. D. 1400
Lomakin, A. D. 839
Loman, A. A. 634
Lomaniec, E. 1027
Lombard, F. F. 633, 643
Lomeniec, E. 513
Longstaff, B. C. 285
López, T. A. 425
Lorenz, M. G. 796
Loschiavo, S. R. 58, 481, 1497, 1548, 1851
Louis-Seize, G. 790
Lovatti, L. 1605
Lovett, J. 872
Lowry, J. B. 1479
Lowry, S. R. 2047
Löyttyniemi, K. 217, 264
Löyttyniemi, R. 264
Lübben, A. 54
Lück, H. 1137
Lugina, Zh. A. 911
Lukács, J. 136
Lund, B. M. 1157, 1838
Lundquist, K. 1342

e, I. S. 1208
an, N. K. 295
kh, E. P. 1220, 1222-1223, 228
likova, N. N. 1226
ch, J. M. 342
n, D. 1530
t, R. K. 31
ns, M. J. 396, 869
M. 282
X. G. 1247
:ario, A. J. L. 369
:ario, E. C. de 369
Bride, K. E. 2025
Cabe, J. B. 1503
Clure, F. D. 148
Clure, P. J. 1840
Clure, T. 1819
Comis, W. T. 2036
Cormick, D. 1963
Curdy, A. R. 557
:Donald, A. B. 1671
Dowell, C. S. 1292
Dowell, D. W. 542
Farlane, G. B. 461
Farlane, J. A. 1931
:Fie, H. J. H. 167
Gaughey, W. H. 1493
Govern, T. P. 1865
Grath, H. 2084
nerney, M. J. 356
nnes, A. 578
:Kenzie, C. R. 346
ckie, D. L. 277
ckie, E. Grant- 1607
Kinley, V. L. 763
Laren, M. E. 1560
Laughlin, J. R. 969
Lean, J. A. 1181
Lean, M. 1190, 1783
Meekin, T. A. 579
Millan, D. C. 336
Nerney, R. 1064
Nicol, R. J. 524
:Rae, I. C. 607
Rae, K. B. 1600
dden, R. H. 542
ddox, I. S. 776, 1374
eda, K. 978
eshima, K. 68
gan, N. 1476-1477
gda, H. A. 1059
gdoub, M. N. I. 581, 1690-691
habir Singh 503
hdi, M. T. 81
heshwari, R. 818
heshwari, R. K. 476, 1577-578
hmood, S. N. 326
hmoud, M. B. 1023-1024
hmoud, S. Z. 580, 1692-693
hmourides, G. 2003
iti, P. K. 1249
jdanac, L. 816
jerus, P. 95
jewski, T. 1126
jor, P. 1133
jumdar, S. K. 370
jumdar, V. L. 1628
jumder, S. K. 744
ksimenko, L. V. 617
ksimenko, N. A. 1199
kundi, R. H. 1939
lama, A. A. 1215
lcolm, S. 594
leszka, R. 2003
lëva, I. L. 1172
lhotra, S. K. 827
lik, O. P. 284
lik, V. S. 973
liky, S. K. Al- 1645
lin, P. 612
linowski, J. T. 1985
llidis, C. G. 726
llory, L. M. 762
l'tseva, O. V. 1356
lysheva, O. N. 1172
nabe, M. 982
ncaş, D. G. 428
Mancilha, I. M. de 1999
Mandal, S. C. 1559
Mandokhot, U. V. 1090
Maness, E. P. 1029
Mankarmde, S. 1311
Manners, J. G. 1109
Manners, K. 230
Manning, J. F., Jr. 2039
Manning, K. 384
Manōlkidēs, K. 605-606, 1154
Manolkidis, C. 605
Manolkidis, K. S. 606
Mansikkamäki, P. 229
Mansingh, A. 1589
Mansour, M. M. 710
Mansour, S. A. 140
Mantis, A. 625
Mapura M., B. 994
Marasas, W. F. O. 1454, 1484
Marcheva, R. D. 1212
Marcuzzi, G. 267
Margaris, N. S. 305
Margaritis, A. 794
Mari, M. 1604
Marijs, J. A. 1101
Marin, V. 1681
Markh, A. T. 510
Marmo, C. A. 1000
Marques, M. A. da S. 1475
Marrakchi, A. El 1085
Marshall, R. T. 1066
Marth, E. H. 191, 411, 706, 1150, 1468
Märtibauer, E. 1723
Martin, C. 330
Martin, J. H., Jr. 1961
Martin, L. A. 8
Martin, R. 151
Martin, W. J. 1967
Martinets, D. V. 839
Martínez, B. 913
Martínez-Flores, R. 1526
Martínez-Jávega, J. M. 1031
Martinez, M. 687
Martinez, M. R. 1887
Martínez T., B. 913-914
Martins, H. M. 427
Martinson, M. M. 2024
Märtlbauer, E. 1144
Maslova, L. A. 675, 1201, 1790
Masson, A. 1153
Masuda, N. 309-310
Matches, J. R. 152
Mathavan, S. 1570
Mathew, G. 1729
Mathews, W. G. 3
Mathur, A. 1578
Mathur, K. C. 939
Mathur, P. N. 1158
Mathur, S. K. 476, 1577-1578
Mathur, S. N. 812
Matsuda, H. J. 1475
Matsuki, Y. 881, 888, 1461, 1514
Matsumoto, S. 410
Matsunaga, H. 664
Matsunaga, T. 1295
Matsuoka, S. 324
Matsuura, S. 982
Matthews, M. E. 9
Mavrommati, C. 401
Maximenko, L. V. 49
Maximov, V. I. 817
Maxino, F. 858
Maycock, R. 53
Mayda, H. A. 1063
Mayer, G. 1010
Mayo, A. Goicoechea 1687
Mead, G. C. 398
Medhi, R. P. 129
Medina, E. B. 1534
Medina, M. 192, 622
Mehanna, N. M. 583
Mehlitz, I. 1061
Mehlman, I. J. 1443
Mehrotra, R. S. 1598
Mehta, V. K. 728
Meijssen, M. J. M. 1434
Meik, J. 530, 1267
Meksongsee, B. 1539
Melikyan, D. A. 911
Melksham, K. J. 118
Melling, J. 407
Mello Filho, A. de T. 1909
Mel'nichuk, G. G. 1238
Menier, J. J. 211
Menon, A. S. 302
Menshikov, N. S. 295
Mensinger, J. D. 1983
Merényi, I. 1712
Mergl, M. 1108, 1138
Merry, J. 1817
Mesquita Lara, F. 944
Mesquita, R. P. de 391
Messina, F. J. 721
Metlitskiĭ, Z. A. 108
Metzner, W. 826
Meussdoerffer, F. 250
Meyers, A. J., Jr. 1835
Mezzalama, M. 509
Michaely, H. 106
Michaux, O. 1726
Micko, M. M. 204
Midon, H. 1655
Mignone, C. F. 1375
Miguez, C. B. 1348
Mihăilescu, N. 529
Mikesell, M. D. 1355
Mikhaĭlov, A. 1777
Mikki, M. S. 1021
Miles, W. F. 52
Milgrom, L. 371
Millán M., B. 994
Millar, J. G. 1878-1879
Miller, D. W. 355
Miller, K. W. 885
Miller, W. R. 527-528
Milliere, J. B. 1119
Milligan, R. H. 213, 1763
Milliken, G. A. 1551
Millim, K. 501-502
Mills, J. 769, 1312
Mills, J. T. 1523
Mills, R. B. 258, 716, 945, 1437, 1542, 1877
Milne, R. 327
Milnes-McCaffrey, L. 157
Mines, R. O. 1974
Minett, W. 286, 740
Mirchev, S. 1741
Mirocha, C. J. 480, 844, 899, 1474, 1525
Mironova, S. N. 1215
Mirzoyan, M. A. 1216
Misawa, N. 2065-2066
Mishchenko, V. F. 1229
Mishra, A. K. 2070
Mishra, S. 2015
Mishra, S. N. 1853
Mislivec, P. B. 1642
Mistry, V. V. 188
Mitchell, F. G. 496, 1010, 1013
Mitchell, G. E. 1103
Mitra, A. 1742
Mitra, S. N. 1191
Mitsui, A. 1295
Miyauchi, T. 1748
Mižik, P. 906
Mizuno, T. 664
Mkhitaryan, K. A. 1002
Mkhize, J. N. 739, 1893
Mladenova, I. 1155
Mo, K. 1845
Moberg, L. J. 862
Mocquot, G. 594
Modi, N. 407
Moebus, O. 1377
Mohamed, M. I. 1908
Mohamed, S. M. 591
Mohammad, A. H. H. 832
Mohammed, M. 132
Mohammed, M. T. 1727
Mohan Singh 1648
Mohd. Hamami, S. 1771
Mohon, B. 69
Moiseev, Yu. V. 1215
Mojžiš, J. 906
Molin, G. 800, 1233-1234
Möller-Bremer, C. 1371
Møller-Madsen, A. 565
Molska, I. 582
Molskness, T. A. 1848
Mondal, K. A. M. S. H. 718
Monib, M. 1314
Montanari, M. 1826
Monte, G. dal 837
Montgomery, S. O. 2012
Monties, B. 786
Moon, J. J. 1139
Moore, A. 451
Moraes Rêgo, A. F. de 59
Morales M., A. R. 1011
Morallo-Rejesus, B. 80, 1901
Mordkovich, Ya. B. 295
Moreno Romo, M. A. 42
Morgan, M. R. A. 444, 1064
Morgan, T. D. 1880
Morgat, F. 1003, 1606
Morichi, T. 399
Morisawa, Y. 664
Morisot, A. 1989
Moro, A. A. 1679-1680
Morozumi, S. 418, 901
Morrell, J. 1796
Morrell, J. J. 222, 644, 1801, 1808
Morrell, R. A. 2037
Morrison, G. J. 171, 558
Mortimer, D. N. 993
Morton, A. 112
Morton, G. 230
Morton, L. H. G. 387, 1940
Moseley, S. L. 874
Moss, M. O. 831, 845
Most, M. M. van der 2022
Mostafa, S. A. S. 1275
Mot, R. de 377
Motkosky, N. 2031
Mottar, J. 185, 1100, 1141
Motte, J. C. 809
Mount, M. S. 1653
Moussa, A. E. 169, 181
Moustafa, M. K. 180, 1094, 1127
Muchmore, F. W. 689
Mueke, J. M. 533
Muhihu, S. K. 1520
Muir, D. D. 563, 1104
Muir, W. E. 77
Mukhopadhyay, P. 1252
Mullen, M. A. 1586
Müller, T. 1486
Mulligan, C. N. 366
Murad, A. K. 1854
Murad, A. M. B. 1256
Murakami, T. 68
Murira, K. K. 1737-1738
Murmanis, L. 198, 631, 635, 1169, 1746
Murmanis, L. L. 1175
Murphy, K. L. 1976
Murray, W. D. 1337
Muruganandan, B. 1481
Musich, E. G. 1237, 1239
Musleh, R. M. 1522
Muthu, M. 281, 744
Myazaki, I. 996
Nabawi, A. El- 1582
Naeem, S. 326
Nagahori, H. 664
Nagalakshmi, S. 805
Nagarajan, R. 1481
Nagayasu, O. 1973
Nagel, W. 1986
Nagler, M. J. 843
Naguib, K. 580, 1692-1693
Nagy, J. 1272
Nahata, M. C. 1218
Nahdi, S. 1465
Nair, G. B. 1058
Nair, K. S. S. 1729, 1766
Najim, L. 504
Nakagawa, Y. 1649
Nakakita, H. 1255
Nakamura, A. N. 1623
Nakamura, K. 2065-2066
Nakano, N. 978
Nakasone, K. K. 210
Nakasuga, T. 987
Nakata, Y. 664
Nakazato, M. 901

Nakkash, S. M. 1021
Nambudripad, V. K. N. 175, 595, 684
Nanda, G. 2070
Nandal, A. S. 973
Nandi, B. 958
Nandris, D. 1758
Nanninga, H. J. 2035
Naoi, Y. 901
Napier, S., Jr. 1819
Naqvi, A. A. 1947
Naqvi, S. A. M. H. 123
Naqvi, S. H. Z. 532
Narasimhan, K. S. 744
Narayanan, C. S. 1244, 1592
Narita, N. 486
Narkis, N. 1960
Natarajan, A. M. 593, 684
Natarajan, C. P. 805
Natarajan, N. 1113-1114
Natour, R. M. 348, 912
Naudé, L. 544
Naudé, R. T. 544
Naumann, H. D. 544
Naumenko, N. F. 1220, 1222-1223
Navarro, S. 725, 1266, 1506
Nawrot, J. 467, 469, 730-731, 734-735
Nazimuddin, S. 924, 1512
Nazma 647
Neal, T. P. 452
Neculce, A. 428
Neerven, A. R. W. Van 314
Neethling, J. B. 1977-1978
Nehra, P. 954
Nelson, M. J. K. 2012
Nelson, P. E. 452, 1454, 1484, 1728
Nelson, T. 65
Nema, D. K. 1611
Nesbitt, W. B. 1029
Nesheim, S. 844, 1450
Netherlands, Netherlands Biotechnological Society NBV 1395
Neuhauser, E. F. 1961
Neut, C. 1433
New Scientist 306, 328, 333
New Zealand, Forest Research Institute 1775
Newbill, M. A. 222, 1808
Neweigy, N. A. 168
NFPA/CMI Container Integrity Task Force 30
Nguyen-The, C. 1613
Ni, Z. Z. 746
Nichols, R. 384
Nickelson, R. 151
Nickerson, J. C. 1265
Nicole, M. 1758
Niedzielska, A. 1123
Niehaus, W. G., Jr. 1530
Nielsen, H.-J. S. 155
Nieuwenhof, F. F. J. 1703
Nieuwenhuizen, M. C. H. 1368
Nigam, J. N. 794
Nikolov, Z. 1697-1698
Niles, E. 440
Nilsson, I. 800
Nilsson, T. 1740
Nimr, A. A. El 1364
Nirenberg, H. I. 1176
Nishijima, M. 410, 421, 901
Nishima, T. 410, 901
Nishimoto, K. 201, 205, 664, 1748, 1810, 1812
Ništiar, F. 906
Ništiarová, A. 906
Nitchals, D. R. 2058
Nitzschke, E. 1711
Niv, S. 1306
Noack, B. 1603
Nockrashy, S. El 580, 1692-1693
Noguchi, M. 1748
Nonveiller, G. 835
Noomen, A. 1101
Norani, A. 1162
Norberg, P. 404
Nørlyng, P. 957
Norman, J. A. 440
Northolt, M. D. 1082
Nortjé, G. L. 544
Norton, J. 1207
Notermans, S. 534, 1439
Noüe, J. de la 330
Nout, M. J. R. 1594
Novello, J. C. 765, 2051
Novogranova, T. I. 1007
Nowakowska, K. 467
Nowicka, D. Pankiewicz- 1566
Nuñez, M. 192, 622
Nur, M. A. 983
Nusrath, M. 1465
Nwosu, V. C. 161
Nwude, N. 1546
Nwunde, N. 938
Nyuksha, Yu. P. 1211
Obafemi, A. 1076-1077
Obawede, K. S. 1673
Oberlander, H. 976
Obermaier, O. 1083
Obrazhey, A. F. 931
Obretenchev, D. 1271
Obuekwe, C. O. 331, 340
O'Connor, F. 594
O'Donnell, R. L. 1607-1608
Odriozola, E. 425
Odunfa, S. A. 1821
Oehlschlager, A. C. 715, 1497, 1516, 1878-1879
Oei, H. L. 757
Offem, J. O. 981
Ofori, J. 1401
Ofuya, C. O. 102
Ogata, J. 1623
Ogawa, J. M. 126
Ogawa, T. 313
Ogawa, Y. 987
Ogihara, H. 179
Ogundana, S. K. 134
Ogundero, V. W. 1483
Oh, Y. J. 890, 1513
Ohms, J. P. 908
Ohtsuki, T. 233
Ojero, M. F. O. 533
Okagbue, R. N. 1518
Okamoto, K. 170
Okamoto, M. 932
Oki, T. 641
Okoro, S. P. A. 1168
Okumura, S. 1748
Oleszkiewicz, J. A. 2009
Olivares Mireles, H. S. 484
Oliveira, D. A. 60-61, 289
Oliveira, M. L. de 59
Oliver, A. C. 383
Olsen, R. H. 1819, 2067
Olutiola, P. O. 135
Olzany Silva, D. 1361
Omar, E. E. 57
Omolo, E. O. 1537
Onan, E. 87
O'Neill, E. J. 2012
O'Neill, I. K. 844
Ono, S. 664
Oosthuizen, W. 544
Orchard, F. 578
Orovan, E. 669
Osipyan, L. L. 617
Osman, N. 455, 1901
Osman, N. B. 1877
Osmani, Z. 1916
Osterholm, M. T. 1116
Osunlaja, S. O. 1483
Osuský, A. 203, 1747
Otani, H. 1372
Othman, F. A. 550
Otjen, L. 200, 209, 1756
Ottolenghi, A. C. 301
Ouakinin, J. S. 427
Oudendijck, E. Van 377
Overseas Development Administration, United Kingdom 822
Özkazanç, A. N. 915
Pacha, R. 638
Pacheco, R. Avilés 66
Packard, V. 238
Packard, V. S. 1720
Padhy, R. N. 820
Padhye, A. P. 948
Padmaja, G. 334
Pain, S. 692
Painter, H. A. 312
Pajni, H. R. 722
Pal, D. 1081
Palma, M. R. 1680
Palma, M. S. R. 1679
Palmer, J. G. 198, 631, 635, 1169, 1746, 1842
Palomes, A. 1467
Palumbo, S. A. 388, 856, 858
Panagiotidou-Mamalouka, V. 1084
Panchenko, L. P. 1347
Pande, A. 1576
Pande, Y. D. 1532
Pandey, G. P. 974, 1264, 1282, 1284
Pandey, N. D. 937, 1937
Pandey, R. S. 119
Pandey, V. 937
Pandurangan, A. G. 1599
Panichsakpatana, S. 1988
Panin, V. V. 1324, 1382
Pankiewicz-Nowicka, D. 1566
Panova, V. F. 783
Papageorgiou, D. 625
Parada, J. L. 873
Parameswaran, N. 1202
Paranjpye, R. 541
Parekh, L. J. 357
Parente, E. 318
Parhad, N. M. 1915
Park, D. L. 482, 1450
Park, H. Y. 587
Park, J. M. 1135
Park, Y. H. 1135
Parker, I. 993
Parkinson, M. 354
Parrish, F. C., Jr. 1065
Parrish, F. W. 464
Parshad, V. R. 274, 1911
Parsons, M. 1614
Parthasarathy, V. A. 129
Pasalu, I. C. 717, 939
Passlow, T. 1540
Paster, N. 918
Paszczyński, A. 705
Patel, G. B. 346
Patel, G. S. 1685
Patel, N. G. 943
Patel, P. D. 22
Patel, R. B. 508, 520
Paterson, A. 1342
Paterson, R. R. M. 2069
Pathak, J. 1433
Pathak, P. H. 1274
Pathak, V. N. 1612
Pathirana, R. A. 234, 682
Patil, M. D. 1915
Patil, R. S. 1040
Patil, R. V. 782
Patourel, G. N. J. le 1558
Patterson, C. G. 1930
Patterson, M. F. 48
Paulillo, A. C. 1475
Paulsch, W. E. 1686
Pavlov, A. 1132, 1231
Pawlosky, R. J. 480
Payne, J. A. 91
Payne, R. G. 1842
Payne, W. L. 874
Pedersen, J. R. 949
Pedersen, P. 1678
Pedro, K. N. Don- 1242
Peek, R. D. 1185
Peeler, J. T. 872, 1435
Pellerin, R. A. 2044
Pellikka, M. 1170
Pelroy, G. A. 541
Peneva, V. 1145
Pennington, L. J. 1482
Pennycook, S. R. 518
Pepeljnjak, S. 479
Pepper, I. L. 1307
Pereira, A. S. 1999
Perelman, A. 918
Perez-Mendoza, J. L. 1359-1360
Pérez, S. Jiménez 1687
Pérombelon, M. C. M. 10
Perrey, H. 668
Perrin, J. 879
Perry, R. 2030
Peru, International Potato Center 1042
Pest Infestation Laborator Denmark 2071
Pestka, J. J. 47, 417
Peteghem, C. H. Van 460
Petelincar, I. M. 996
Peterkin, P. I. 195, 403
Peters, R. 1664
Peterson, M. E. 541
Peterson, R. E. 1515
Peterz, M. 404
Petkov, R. 1079
Petkova-Bocharova, T. 88
Petríková, D. 997
Petrosyan, R. A. 1214, 12
Petrov, V. G. 1220, 1222-1223, 1228
Petrowitz, H. J. 228
Petruccioli, M. 2034
Pettipher, G. L. 130
Petushkova, Yu. P. 628-62 1226
Petzinger, G. 1450
Peyresaubles, R. 1776
Pfeiffer, R. 897
Pfleger, F. 623
Pflieger, F. 221
Phan, C. T. 1050
Phillips, J. D. 563, 576, 1 1721
Phillips, M. P. 71
Phillips, T. D. 1471
Philogène, B. J. R. 738
Picard, G. 330
Picard, G. A. 299
Picken, G. B. 694
Pico, R. F. 2054
Pierce, A. M. 715, 1516, 1879
Pierce, H. D., Jr. 715, 149 1516, 1878-1879
Pierson, M. D. 555, 2075
Pietri, A. 571
Pietzsch, O. 572
Pignatello, J. J. 2024
Pikelis, S. 556
Pilando, L. S. 1636
Pilloud, R. 1046
Pilyashenko-Novokhatnyu, I. 1224
Pimentel, M. L. 59
Pinheiro, A. J. Rezende 1
Pinniger, D. B. 824, 1260, 1890
Pino, M. P. del 2056
Pipes, W. O. 1293
Piton, C. 1118
Pitt, J. I. 385-386, 1404, 1
Pittman, C. W. 452
Piva, G. 571
Pivnick, H. 594
Pizarro, D. R. 1993
Pizzi, A. 669, 1184, 1187, 1799, 1802
Place, I. C. M. 1398
Plackett, D. V. 1210, 1800
Plant Quarantine Branch, Commonwealth Departm of Health, Australia 262
Plasse, R. 1050
Plattner, R. D. 955
Playne, M. J. 2016
Poernomo, S. 183
Pogrebnyak, L. I. 931
Pohland, A. 422
Pohland, A. E. 1642
Pohland, F. G. 323
Polasa, H. 1452
Pol'kin, S. I. 1324, 1382
Polo Villar, L. M. 190
Pometto, A. L., III 1733
Poncini, G. F. 88
Poncini, L. 88
Pope, A. R. 1272
Porter, J. E. 1496

erfield, M. L. 452
ošil, O. 487
, L. S. 278, 1950
e, L. M. 32
chke, M. 1037
er, N. N. 245
s, D. 1560
s, M. F. 1930
arkova, G. S. 783
o Lora, R. 190
hu, V. K. K. 1244
a Neto, W. L. 707
dzyńska, A. 454, 732-733
kash, A. 939, 1286, 1531, 533
kash, O. 516
ad, B. 1620
ad, G. 567
har, M. 115
ap Singh 1768
koppová, J. 1110, 1151, 715
usky, D. B. 45
nlata Singh 1459
ly, A. H. 144
s, J. W. 1550
ton, A. F. 1800
e, U. del 1096, 1575
vett, P. F. 838, 1931
e, N. R. 292, 1290
e, T. H. 572
epskil, E. A. 839
chard, P. H. 2012
kop, A. 348
sky, D. 113
Z. L. 468
key, D. J. 167
an, Z. 1376
an, M. S. 499
naiah, K. C. 1573
ovac-Velikonja, A. 895
wadaria, T. 1478
ch, D. J. 480
ey, P. L. 1617
hpamma, P. 964, 966-967
ri, R. B. 988
snel, L. B. 20
ckenden, J. 2052
llardet, P. 1146
nta, M. L. 877
ntanilla, J. A. 254
ntavalla, S. 173
lte, G. D. van 1167
o, H. G. El 1095
bie, C. J. 54, 1454
bindra, R. J. 727
binovitch, L. 391
zkowski, J. 638
lić, B. 487-488
loi, V. 529
lwan, H. S. A. 1582
thavan, G. S. V. 1050, 054
man, H. A. Abdel- 269-70
, A. K. 1884
, R. P. 1652
, R. S. 1511
ak, R. L. 1553
asekaran, B. 922, 1250
endran, S. 744, 1254
orhia, G. S. 1081
n, B. P. 417
n, V. 1633
nade, C. 1111
nakrishnan, C. V. 357
nan, K. V. 138-139
nanjaneyulu, G. 1702
nantanis, S. 854
nchandani, N. P. 1020
nchandra 953
nírez, C. 208
nos Cartagena, M. del C. 2
nsay, B. A. 2060
ndhawa, K. S. 1648
ndle, P. E. 384
nganathan, B. 177, 564, 93, 1099, 1689
ngaswamy, J. R. 744
o, A. M. 2044
Rao, B. H. K. M. 940
Rao, C. S. 940
Rao, J. 1531, 1533
Rao, J. M. 805
Rao, P. V. 447
Raoof, M. A. 516
Rapp, P. 1338
Ratcliffe, H. D. 348
Rathore, A. K. S. 1778
Ratnadass, A. 1647
Rauschmaier, R. 1358
Rautiainen, L. 827
Ravilly, F. 1209
Rawaf, S. 1661
Rawal, B. D. 1218
Rawal, R. D. 125
Rawash, M. A. 90
Rayman, M. K. 19
Rayner, A. D. M. 648-650, 1173
Razdan, M. 1449
Rea, M. 594
Read, R. B., Jr. 89, 148
Reagan, J. O. 549, 553
Realini, M. 1823
Rebhun, M. 1960
Reddy, B. N. 1465
Reddy, C. A. 1241, 2067
Reddy, C. V. 447
Reddy, K. R. 378-379
Reddy, K. V. S. 1537
Reddy, N. S. 1689
Reddy, P. S. 447
Reddy, R. R. R. K. 1097
Reddy, V. R. 447
Reddy, Y. K. 1097
Redhead, S. A. 651
Reeleder, R. 1054
Rees, D. P. 1261, 1267
Rêgo, A. F. de M. 59
Reichmuth, C. 1273
Reid, I. D. 341, 1761
Reid, J. C. 1589
Reidelshöfer, A. 1093
Rejesus, B. Morallo- 80, 1901
Renaud, A. M. 14
Renner, G. 1942
Renterghem, R. van 1141
Renuka, B. R. 1295
Renwick, J. A. A. 721
Report, Centre for Overseas Pest Research, UK 823
Resnizky, D. 1014
Rezende Pinheiro, A. J. 1361
Rhodes, P. 20
Ribéreau-Gayon, P. 96
Richard, J. 624, 1117-1118, 1120
Richard, J. L. 1462
Richardson, B. J. 231
Riding, R. 2083
Riehl, M. 1093
Riesenfeld, G. 889
Riet, J. Van't 802
Riet, W. B. Van der 494
Rigby, D. J. 799
Rimmington, A. 320
Ringpfeil, M. 1997
Rintelen, J. 448, 917
Rio, B. 1758
Ripp, B. E. 1501
Risse, L. A. 527-528
Ritter, G. 1743
Ritter, H. 668
Rittmann, B. E. 1981
Rivas, M. 1679-1680
River, B. H. 1797
Rizzo, A. 39
Roach, J. A. G. 1642
Robbs, N. K. 11
Robbs, P. G. 11
Robert-Baudouy, J. 698
Roberts, D. 186, 572, 602
Roberts, D. P. 1653
Roberts, P. B. 1428
Roberts, R. G. 1584
Roberts, T. A. 1067, 1840
Roberts, W. T. 1217
Robertson, J. A. 1584
Robertson, L. A. 768, 803
Robic, D. 1160
Robinson, J. H., Jr. 1969
Robinson, R. A. 1116
Robinson, R. W. 766
Roby, J. J. 1607-1608
Rochanda, L. 1478
Roche, R. 1571-1572, 1859
Rodrigues, U. M. 1688
Rodrigues, Z. A. 59
Rodríguez, A. 1588
Rodriguez-Amaya, D. B. 1469
Rodriguez Ferri, E. 1131
Rodríguez, H. 338
Rodriguez, I. R. 1130
Rodriguez, J. G. 1930
Rodríguez, L. Domínguez 1131
Rodríguez, M. 1588
Roffael, E. 1202
Rogol, M. 389
Rohani, M. Y. 465
Romanenko, V. I. 787
Romer, T. R. 1460
Romero, A. 1443
Romond, C. 1433
Rood, H. D., Jr. 1722
Rose, S. A. 23
Roseland, C. R. 1880
Rosen, J. D. 433
Rosen, R. T. 433
Rosenbaum, H. 75, 286
Rosenthal, R. A. 1219
Ross, A. 1335
Rossi, J. 594
Rossman, L. A. 1968
Rossmoore, H. W. 297, 1943-1944
Rot, I. 121
Rothenberg, P. J. 18
Rothman, D. 389
Roubalová, V. 860
Rouhbakhsh-Khaleghdoust, A. 1426
Rouse, C. D. 452
Rousseau, D. M. 460
Roux, F. 96
Rowe, M. T. 1708
Rowell, R. M. 1793
Roy, A. K. 1630
Roy, A. N. 478, 526
Roy, D. 723
Roy, S. 1280
Rozanova, E. P. 1225
Rozich, A. F. 2028
Rubin, H. E. 176
Ruckdeschel, G. 1942
Ruddick, J. N. R. 1798
Rudenko, E. L. 510
Ruel, K. 636
Ruetze, M. 1774
Rümann, J. 1772
Rup, P. J. 1894
Russell, E. K. 1974
Russell, G. E. 1643
Russell, I. 2053
Russell, N. J. 1353
Rusul, G. 1468
Ryan, T. B. 452
Rydell, R. 827
Ryndina, L. V. 1177
Ryvarden, L. 1406
Saad, N. H. 1043
Saakyan, K. A. 1216
Sabbour, M. M. 1692
Sabey, B. R. 1309
Sadana, J. C. 782
Saddik, M. F. 70, 538
Saddler, J. N. 790, 1333, 1336
Saeng-on, B. 971
Safaryan, Z. S. 1214
Sahab, A. F. 977
Sahm, H. 1305
Saibaba, M. 1831
Saito, A. 324
Saito, K. 901, 1296, 1300-1303
Saito, Y. 900
Sakai, T. 886, 1649
Sakamoto, T. 664
Saksena, S. B. 1657
Sala, N. 1467
Salam, M. H. Abd-El 611
Saleem, M. A. 280, 1276-1277
Saleem, R. 169
Saleem, R. M. 181, 1683
Saleh, S. M. 1268
Saliba, N. S. 1788
Salita, A. A., Jr. 1166
Salman, A. J. 912
Salmeen, I. 1819
Salunkhe, D. K. 495
Salunkhe, G. N. 946, 1651
Samolada, M. 605
Sampaio, V. R. 1622
Samragy, Y. A. El 581, 1690-1691
Sams, C. E. 114
Samson, R. A. 703, 1404
Samsudin, A. 465
Samuel, A. 230
Samuel, R. 1244
Sanchez, M. G. C. 1038
Sanchis, V. 1467
Sanders, T. H. 979
Sanderson, D. B. 77
Sandhu, D. K. 1331, 1846
Sandlant, G. R. 1764
Sándor, G. 1487-1488
Sandvol, L. 1508
Şanlı, Y. 915
Šano, H. 309-310
Santamarina, P. 1467
Santana-Castillo, L. 1360
Santos, E. C. dos 1140
Santos, F. L. 1902
Saplina, V. I. 1172
Sarhan, H. R. 610, 1676
Sarin, K. 952, 954
Sarjono, J. 1267
Sarver, E. W. 430
Sasakawa, M. 987
Sassanarakkit, S. 1988
Sassoon, A. 759
Sato, K. 179
Sattigi, H. N. 1545
Satyaprasad, C. H. 1627
Saud, H. M. Al- 1854
Saull, M. B. 769, 1312
Saunders, G. K. 452
Saunders, M. F. 442
Sauphanor, B. 1647
Savage, P. 286, 740
Savell, J. W. 548
Sawaf, S. D. Al 1683
Sawaya, W. N. 550
Saxena, A. 1657
Saxena, P. N. 1884
Saxena, S. 1884
Saxena, S. C. 1883-1884, 1898
Sayem El Daher, N. 156
Sayler, G. S. 359
Schade, J. E. 43-44
Schaefer, O. 2042
Schaik, A. C. R. van 1006
Scheffer, T. C. 1762
Scheffers, W. A. 2068
Scheiding, W. 1335
Scherer, A. 541
Schiffmann-Nadel, M. 106, 513, 1027
Schimz, K.-L. 337
Schink, B. 316
Schipper, M. A. A. 54
Schirra, M. 514, 1019
Schlitzer, R. L. 1219
Schmidt, E. L. 224, 232, 1195
Schmidt, H. E., Jr. 2037
Schmidt, R. 898
Schmidt, S. K. 304
Schmutzenhofer, H. 1403
Schneider, H. 2003, 2019
Schöber, B. 1451
Schocken-Iturrino, R. P. 1475
Scholefield, J. 726
Scholten-Koerselman, H. J. 779
Scholz, E.-G. 1052
Schöne, B. 1366
Schothorst, M. van 14, 572
Schouten, S. P. 1006, 1048
Schoutens, G. H. 1368
Schrem, J. 1122
Schröder, M. J. A. 1719

Schroeder, L. L. 1028
Schug, A. 1305
Schügerl, K. 1335
Schülein, M. 349
Schuller, P. L. 844
Schulz, G. 227
Schulze, K. 1658
Schuster, M. 896
Schütz, M. 1366
Schwab, A. H. 89, 148
Schwach, T. S. 237
Schwartz, R. D. 810, 1379
Schwenk, S. 100, 992
Scienza e Tecnica Lattiero-Casearia 2074
Scott, C. D. 2073
Scott, K. J. 1719
Scott, P. 844
Scott, P. M. 457, 844, 894, 904, 1455
Scott, P. N. 956
Scudamore, K. 435, 450
Scudamore, K. A. 440
Scurrah, M. 138
Seal, K. J. 234, 682, 1407
Sealy, L. 132
Sealy, L. H. 500
Sechter, I. 389
Seehann, G. 645
Segar, D. A. 1987
Segar, K. 1660
Segura, J. 325
Seibel, E. 1457
Seidel, K. 1964
Seifelnasr, Y. E. 945
Sejiny, M. J. 456
Seligmann, R. 594
Semenov, S. A. 1215
Semichaevskii, V. D. 1238, 1388, 1735
Senanayake, U. M. 1869
Sengupta, R. 1252
Sengupta, T. 1252
Serafim, M. B. 408
Serpokrylov, N. S. 1347
Serralheiro, M. L. 877
Seshadri, R. 805
Seshu Reddy, K. V. 1537
Sethi, G. R. 728
Sethi, R. P. 1369
Severin, W. P. J. 1440
Sewell, A. M. 1489
Sezgin, E. 87
Shabunina, T. I. 1315
Shahani, K. M. 2047
Shaker, H. M. 2018
Shaler, T. A. 2023
Shales, S. W. 243
Shamoun, S. F. 642
Shamshad, S. I. 988
Shanker, S. 974, 1264, 1282, 1284
Shannon, G. M. 955, 1515
Shannon, W. 934
Sharaf, M. A. 1995
Shareef, A. Y. 1672
Sharkey, P. J. 1025, 1621
Sharma, A. D. 1341
Sharma, H. S. S. 630
Sharma, J. P. 84
Sharma, K. 952, 954
Sharma, M. N. 1191
Sharma, R. B. 478
Sharma, R. K. 1026
Sharma, S. L. 1593
Sharma, V. K. 950-951
Sharma, Y. 1263, 1936
Sharom, M. S. 525
Sharp, W. 1508
Sharpe, A. N. 195, 403
Sharples, R. O. 1005
Shaw, B. G. 167
Shay, L. K. 1996
Shazali, M. E. H. 947, 1544
Sheets, P. J. 1383
Shehata, A. E. 581, 1690-1691
Sheikh, A.-S. 93
Shelke, S. S. 1651
Shell International Chemical Company 275
Shemyakina, T. M. 628-629
Shen, C. P. 1907
Shen, M. L. 474
Shepherd, M. J. 414, 458, 615, 891, 972, 993, 1147
Sher Singh 1644
Sherard, R. C., Jr. 158
Sheridan, J. E. 1392
Sherrard, J. H. 1974
Shiang, M. 1334
Shibiny, S. El 583
Shieh, J. 1069
Shields, M. S. 359
Shimizu, K. 700
Shinde, P. A. 948, 1626
Shinmoto, M. 641
Shiota, C. M. 944
Shirvani, M. 146
Shivaramaiah, H. N. 1254
Shomer, I. 99
Shone, C. 407
Shotwell, O. L. 878, 955, 1515, 1527
Shpak, B. 389
Shteinberg, B. I. 681
Shub, G. M. 1357
Shukla, D. N. 119
Shukla, R. M. 1552, 1864, 1896-1897
Shuler, M. L. 693
Shutov, G. M. 1770
Sidhu, M. S. 1846
Sighamony, S. 1916
Sikora, L. J. 1297
Sikström, E. 1832
Silas, J. C. 867
Silhacek, D. L. 976
Silva, D. Olzany 1361
Silva, P. 848
Silvestri G., R. 913-914
Sim, T. F. 37
Sim, T. S. 37
Simanca, M. E. 1571-1572, 1859
Simard, R. E. 156, 162, 164, 1316-1317
Simizu, Y. 179
Simkins, S. 304
Simonaitis, R. A. 679
Simonova, G. A. 1347
Simonsen, B. 394
Simpson, B. W. 71
Sims, G. K. 2011
Sinclair, E. R. 6, 263, 714
Sindhu, R. S. 360
Singh, A. P. 1739
Singh, B. 1283
Singh, B. P. 512
Singh, J. 1990
Singh, J. P. 1644
Singh, K. M. 961, 965
Singh, Kishan 2049-2050
Singh, L. 1369
Singh, M. 503, 1648
Singh, O. P. 1536
Singh, P. 1768
Singh, R. 1535
Singh, R. N. 1310
Singh, R. S. 115, 1099, 1640
Singh, S. 1563
Singh, S. P. 512, 1949
Singh, T. 941
Singh, T. G. 1627
Singh, Y. P. 56, 1283
Singh, Z. 284, 973
Singmin, M. 669
Sinha, B. P. 478
Sinha, K. K. 1459, 1631
Sinha, R. N. 73-74, 77, 1523, 2081
Sirandhana, B. S. 1458
Sive, A. 1014
Sizemore, R. K. 153
Skakun, T. O. 1324, 1382
Skelley, G. C. 158
Skryabin, G. K. 1345
Slavchev, G. 871
Slegers, G. A. 460
Slininger, P. J. 1332
Slottová, A. 1149
Sly, L. I. 368
Slyusarenko, N. F. 1299
Slyusarenko, T. P. 1315
Smedt, J. de 1441
Smilanick, J. L. 866
Smiley, R. L. 1906
Smirnova, G. F. 1349
Smith, D. G. 1967
Smith, G. C. 548
Smith, J. E. 38, 811, 845
Smith, J. F. 384
Smith , J. H. 1308
Smith, L. B. 545, 752, 1491, 1507
Smith, M. 1442
Smith, R. 151, 1986
Smith, R. E. 331, 340
Smith, R. H. 473, 947, 1544, 1876, 1886
Smith, R. S. 1804
Smith, S. M. 1808
Smithwick, C. M. 523
Smoczyński, S. 626, 1674
Smulders, F. J. M. 551-552, 1072
Sneck, T. 827
Snyder, P. O. 9
Soares, L. M. V. 1469
Sobczak, E. 2033, 2041
Society of Food Hygiene Technology 1258-1259, 1396
Society of Food Hygiene Technology, United Kingdom 824
Soderstrom, E. L. 1929
Sodhi, H. S. 1480
Soekarto, S. T. 983
Søgaard, H. 859, 1678
Sohi, H. S. 498
Solberg, M. 278
Solfrizzo, M. 1143
Soliman, Z. A. 269-270
Solomon, B. 1245
Solomon, H. M. 31
Solomon, N. A. 1409
Solov'ev, V. A. 1172
Somers, E. B. 554, 1152
Sommer, N. F. 13, 40, 496, 1013, 1646
Sommers, L. E. 2011
Sonawane, C. S. 1626
Sondossi, M. 297, 1943-1944
Soni, G. J. 1695
Sonnenmoser, H. 621
Sood, A. 1369
Sorlini, C. 1823
South Africa, National Timber Research Institute 2078
Souty, M. 1613
Souza, H. M. de 1140
Souza Leão Veiga, A. F. de 59
Sowers, M. A. 1297
Spalding, D. H. 528
Sparling, A. B. 2009
Spaulding, P. L. 1663
Speckhard, M. W. 1152
Speirs, W. E. 1865, 1885
Spencer, D. M. 384
Sphon, J. 422
Sphon, J. A. 1450
Spiegelenberg, W. M. 489
Spilman, T. J. 1875
Spilmann, J. R., Jr. 409
Spiring, F. 1667
Spitler, G. H. 117
Splittstoesser, D. F. 991
Spotts, R. A. 506-507, 1610
Spreekens, K. J. A. van 1659
Springle, W. R. 1822
Spruit, C. 1006
Spurgash, A. 556
Sreedharan, V. P. 1592
Srinath, D. 853
Srinivasan, M. R. 566, 1086, 1088-1089
Srinivasan, R. A. 595, 1714
Srivastava, A. S. 1864, 1897
Srivastava, J. L. 1264, 1279, 1282
Srivastava, K. P. 1538
Srivastava, P. K. 1511
Srivastava, R. A. K. 812
Srivastava, R. C. 729
Srivastava, R. K. 490
Srivastava, S. K. 812, 974
Srivastava, U. S. 729
Srivastava, V. J. 1983
Stables, L. M. 933
Stack, M. E. 1642
Stadhouders, J. 1082
Stahr, H. M. 897
Stakheev, I. V. 1313, 1319, 1363
Stalker, D. M. 2025
Stamman, E. 1987
Stammler, C. 66
Stannard, C. J. 685
Stark, R. W. 834
Starovoitov, I. I. 1350
Startin, J. R. 414, 458
Stasińska, B. 467
Stauffer Chemical Co. 775, 810
Stavenuiter, J. F. C. 51
Stead, D. 1837
Steinmeyer, S. 930
Stekelenburg, F. K. 1666
Steller, S. D. 1278
Stenström, I.-M. 149
Stepanyuk, B. B. 990
Stepanyuk, V. V. 1347
Stephenson, T. 2030
Stepien, Z. 1881
Stern, N. J. 18, 160, 546, 2075
Stersky, A. K. 1662
Stewart, A. W. 8
Stewart, C. S. 367
Stier, D. A. 986
Stiffler, D. M. 548
Stimson, W. H. 38
Stino, M. N. 1043
Stockar, U. von 1365, 1373, 2042
Stockenström, S. 426
Stoloff, L. 844
Stone, J. M. 18
Stössel, P. 1581
Stott, J. F. D. 1825
Stouffer, J. R. 1070
Stover, E. L. 2055
Stratton, S. C. 1979
Straube, G. 2026-2027
Streisel, R. C. 1165
Strickland, C. 1470
Stringer, M. 1661
Strom, P. F. 777-778
Stromberg, V. K. 1653
Stroshine, R. 462
Stubblefield, R. D. 955
Stubbs, M. R. 1890
Su, H. C. F. 1288, 1863
Suarez, E. J. 1130
Suárez Fernández, G. 42, 1
Subramanyam, B. 923
Subryan, L. M. 1560
Sudha, V. 593
Sudheendrakumar, V. V. 17
Südi, J. 574-575
Suffet, I. H. 1293
Sugihara, K. 886
Sugiura, Y. 1513
Suhren, G. 575, 590, 598-59
Sujatha, A. 1573
Sultan, N. E. 1690-1691
Sultana, K. 1732
Sulthoni, A. 1178
Sumbali, G. 1598
Summers, N. A. 678
Sumner, S. S. 1152
Sun, M. 596-597
Sundlof, S. F. 1470
Suplicy Filho, N. 996
Suresh, E. R. 101
Surevich, V. 1813
Surewicz, W. 1813
Surma-Slusarska, B. 1813
Surma-Slyusarska, B. 1813
Surovtseva, E. G. 1325-1326
Suryanarayanan, T. S. 1599
Süssmuth, R. 1457
Sutarma 183
Sutherland, J. B. 653
Sutherland, J. W. 1504

ster, H. P. 1948
ston, D. L. 379
ston, H. C. 1428
ton, M. D. 904
uki, H. 1372
uki, I. 399
uki, M. 486
uki, T. 709, 813
rnik, V. I. 1349
stova, I. D. 1387
vine, G. 118
nson, S. P. 1722
rtzentruber, A. 89, 148
ncer, D. 6, 75, 263, 285-288
ngs, J. 1635
tzerland, Eidgenössische ebensmittelbuch-Kommission 614
tzerland, LIGNUM 1794
anco, C. Y. Lim- 1902
ia, V. L. 1471
non, B. 1592
chin, S. 1237
chin, S. A. 1239
bó, B. 1682
bo, R. 1662, 1667
bo, R. A. 19
kály, S. 1122
rka, J. 136
biotko, K. 1074
ta, G. 1124
nidt, A. 218
eyn, J. 1718
bata, S. 410
busa, R. S. 1824
deo, A. R. 1328
lenuma, M. 1296, 1300-1303
ier, M. A. 1732
iir, N. M. 1522
nter, F. H. 1744
i, S. K. 1612
agi, K. 315
ahashi, F. 68
kano, T. 1562
keda, K. 813
n, M. K. 1206
nblyn, N. E. 1401
nboura 1056
i, A. G. 1206
i, K. H. 695
i, S. C. 1618
iabe, I. 1973
iaka, T. 881, 888, 890, 932, 1461, 1513-1514
itaoui-Elaraki, A. 298, 485, 1085
asenko, N. F. 1299
goński, Z. 1731
ca, G. 536
tayre, G. M. 299
arov, P. G. 510
ik, S. M. 807
itorus, T. E. 1381
vfeek, N. 580, 1692-1693
vfik, K. A. 456, 806
lor, J. M. 1189
lor, S. L. 554, 1152
hobanoglous, G. 1971
bach, R. 930
xeira, M. A. 1999
fford, D. R. 3
b, T. 37
otia, T. P. S. 1552, 1896
auchi, T. 1302
nström, A. 1233-1234
plan, G. 573, 1144, 1684, 1723
racina, F. 643
ry, L. A. 694
sone, S. 594
ssier, B. 704
ber, M. 589, 1377
nissen, M. J. 2007
wari, D. K. 128
wari, H. K. 1369
wari, M. C. 1191, 1778
wari, R. P. 815
acker, C. 1662
ikar, R. P. 1696
Thakur, D. P. 1644
Thakur, M. L. 654
Thakur, R. K. 1903
Thanei-Wyss, U. 373
Thayer, D. W. 858, 1069
Theodore-Gandi, B. 1661
Theriault, R. 1050
Thiel, P. G. 426, 1454, 1484
Thiery, D. 79
Thind, B. B. 400, 1269, 1472
Thirupathaiah, V. 1627
Thite, A. N. 1580
Tho, Y. P. 1162
Thoma, M. 1335
Thomas, C. E. 1192
Thomas, J. M. 1958
Thompson, B. K. 1464
Thompson, D. P. 1240, 1952
Thompson, J. 1815, 1866, 1868, 1870-1871
Thompson, N. E. 1449
Thomson, J. E. 15, 172
Thontadarya, T. S. 1545
Thornburg, W. 1623
Thörnqvist, T. 1811
Thornton, I. R. 1025, 1621
Thornton, J. D. 1754
Thorpe, C. 422
Thorpe, C. W. 844
Thorpe, G. R. 1504
Tiebach, R. 1061
Tiecco, G. 187
Tiedje, J. M. 2029
Tigaud, S. 1111
Tikhova, D. 1145
Tilak, S. T. 1831
Tilton, E. W. 1505, 1938
Timakova, T. M. 787
Timmermans, P. A. 1446
Timms, M. 1998
Ting, W.-T. 402
Tips, P. D. 572, 1664
Tiunova, N. A. 817
Tjahjono, L. S. 1478
Toci, R. 344
Todd, E. C. D. 19, 390, 393, 1424, 1662, 1667
Tokimoto, K. 1174
Tokita, F. 1372
Tolle, A. 559, 598-599, 620
Tomlinson, D. L. 1039
Topper, K. F. 1309
Torre, G. della 616
Torres Vitela, R. 568-569
Toti, L. 1425
Tournier, J. P. 1989
Toussoun, T. A. 1454
Trakhtenberg, S. I. 681
Trandaf, F. 536
Trauba, R. L. 1517
Tredici, M. 808
Trematerra, P. 1855
Trenchard, H. 157
Trenholm, H. L. 45, 904, 956, 1464
Tripathi, R. A. 1937
Tripathi, S. C. 1949
Trojanowski, J. 351
Tropical Development and Research Institute, United Kingdom 822, 838
Tropical Products Institute, United Kingdom 822
Trost, M. 772
Trucksess, M. W. 1450
Tsai, L. T. 474
Tseng, T. C. 413
Tsuboi, S. 902, 980
Tsuchii, A. 813
Tsunoda, J. 1812
Tsunoda, K. 205, 1810
Tsvetkov, D. 1271
Tsygankov, S. P. 1299
Tu, C. W. 2048
Tucek, F. 1975
Tuite, J. 462, 1474
Tulus, L. 184
Tutel'yan, V. A. 49, 617
Tutour, B. le 485
Twedt, R. M. 1661
Twomey, D. G. 277
Tyagi, D. K. 1941
Tyagi, R. P. S. 941
Tyczkowska, K. 1148
Tyczyńska, B. 1123
Tyrygina, G. I. 804
Tzanetakēs, N. 605
Tzanetakis, N. 605
Uchiyama, M. 900
Uchiyama, S. 900
Udagawa, S. 486
Udagawa, S. I. 255, 702
Ueno, Y. 829, 844, 881, 888, 890, 1461, 1513-1514, 1561
Uhm, T. B. 372
UK, British Standards Institution 1188
Ullasa, B. A. 125
Umezawa, T. 347
Umięcka, L. 1032
Umoh, J. U. 1673
Umoh, V. J. 1673
Umphres, M. B. 1954
Ungaro, M. T. S. 996
Unger, M. 1307
Unger, W. 239, 1769
Ungsunantwiwat, A. 716
United Kingdom, Centre for Overseas Pest Research 822
United Kingdom, Overseas Development Administration 822
United Kingdom, Society of Food Hygiene Technology 824
United Kingdom, Tropical Development and Research Institute 822, 838
United Kingdom, Tropical Products Institute 822
Unterman, R. 1329
Upadhyaya, G. 526
Upadhyaya, J. 1620
Updegraff, D. M. 2002
Upitis, A. 329
Upsher, F. J. 756
Uradziński, J. 1718
Urban, E. 1609
Uritani, I. 1649
Ursing, J. 1234
USA, American Society for Testing and Materials 1411-1422
USA, American Wood-Preservers' Association 1397
Usborne, W. R. 26
Ushiyama, H. 410
USSR, Gosstandart 1781
Utikar, P. G. 948, 1626
Utley, D. 53
Vacharotayan, S. 1311, 1988
Vairo, M. L. 97
Valenzuela, M. González 1571-1572, 1859
Van Egmond, H. P. 51
Van Hoof, J. 1071
Vanderiet, S. J. 392
Vanderzant, C. 548
Vanoušková, S. 860
Vanselow, D. G. 1478-1479
Ványi, A. 1487-1488
Varadaraj, M. C. 175
Vardell, H. H. 1505, 1938
Varma, R. V. 1729
Vasil'eva, G. K. 1325
Vassiliadis, P. 401, 572
Vázquez Boland, J. A. 1131
Veiga, A. F. de S. L. 59
Veillet-Poncet, L. 1119
Velikonja, J. 895
Veliky, I. A. 2040
Velkov, L. 1601
Venkataramani, V. V. 1158
Venkayya, D. 1097
Vennes, J. W. 761
Venugopal, M. N. 1660
Verachtert, H. 377
Verigin, V. G. 1879
Verma, O. P. 1612
Verma, S. P. 1283
Verma, T. 1423
Vermaak, C. J. 745, 1272
Verweij, A. 429
Verwey, E. W. 765
Vestal, J. R. 763
Vetro, R. 1623
Viana, P. A. 1541
Vicente, M. M. A. 391
Vick, C. B. 1807
Vick, K. W. 976
Videla H., S. 362
Videlov, Kh. 1741
Vidon, D. J. M. 850-851, 857, 870
Vieira Guimarães, W. 1361
Vieira, M. M. 1694
Vienne, P. 1365, 1373, 2042
Viesturs, U. 329
Viet, C. 349
Vihavainen, T. 827
Viitanen, H. 662
Vilder, J. de 1141
Vilela, M. A. P. 1140
Viljoen, J. H. 745, 1272
Villar, L. M. Pollo 190
Vimala, V. 964, 966-967
Vincent, P. 1111
Vir, D. 1022, 1026, 1633
Viraraghavan, T. 1298
Visconti, A. 472, 899, 1143
Visser, I. J. R. 1665
Vitela, R. Torres 568-569
Vlachos, I. 1112
Voerman, S. 283
Vogel, M. C. 669
Vogel, T. M. 2020
Vogels, G. D. 767, 2014
Voget, C. E. 1375
Vokou, D. 305
Volfova, O. 338
Volk, V. V. 1383
Vorob'ev, V. F. 108
Vyas, N. L. 1634
Vyas, S. H. 1702
Waals, C. B. van der 1703
Waart, J. de 2022
Waczkowski, W. 1378
Wada, H. 1311
Wada, T. 902
Waddell, M. S. 1553
Waes, G. 178, 185, 594
Wagner, A. 1682, 1712-1713
Wagner, F. 1338
Wagner, M. K. 27, 1668, 1839
Wainwright, M. 1346
Waitha, M. 88
Wajland, M. 199
Waks, J. 513, 1027
Wälchli, O. 661, 1803
Waldie, P. 1789
Waldner, R. 250
Walker, H. W. 1065
Walker, L. P. 2044
Walker, R. A. 396, 869
Walker, S. J. 1709
Wallace, D. J. 400, 1269
Wallbank, B. E. 287
Wallnöfer, P. R. 896
Wallwork, M. A. 1147
Walt, W. H. Van der 494
Walter, M. V. 761
Walters, M. J. 1057
Walton, D. W. H. 680
Wan, C. K. 1018
Wang, C. C. 1805
Wang, C. J. K. 643
Wang, E. I. C. 204
Wang, Q. 1619
Wang, S. Y. 1070, 1806
Warburton, D. W. 195
Warchalewski, J. R. 467
Wardowski, W. F. 521
Ware, G. M. 1725
Warner, R. M. 45
Warsy, J. D. 570
Wart, M. Van 15
Warth, A. D. 25
Watanabe, N. 28, 962
Waterford, C. J. 1933
Waters, W. E. 834
Watson, D. B. 294
Watson, D. H. 1453
Watson, G. K. 1352-1353

Watson, J. E. 1307
Watt, J. C. 1728
Watters, F. L. 713, 1921, 2081
Wauke, T. 418, 901
Ważny, J. 640
Wazny, J. 1754
Weber, A. S. 1971, 1974
Weber, M. 1160
Weber, R. 930, 1061
Webster, G. R. B. 1548
Wee, Y. C. 695
Weerasinghe, B. 532
Wegecsanyi, M. 75
Wegner, G. H. 1996
Wehling, R. L. 949
Wei, C. I. 17
Weidner, H. 755, 1951
Weilenmann, H. U. 2021
Weinberg, Z. G. 1060
Weis, S. A. 999-1000
Weissman, S. 889
Wells, J. M. 91
Wells, S. L. 247
Welsh, F. W. 2040
Welsh, P. C. 153
Wentworth, R. L. 1995
Wentz, B. A. 89, 148, 1443
Westlake, D. W. S. 2031
Westlake, K. 875
Weta 852, 1899-1900
Wetzel, A. 1294
Wetzel, D. L. 949
Whalen, P. J. 2047
Wheatley, C. 424
Wheeler, J. 1955
Whelehan, O. P. 1067
Whitaker, T. B. 979
White, C. H. 1707
White G. F. 799
White, G. F. 1352-1353
White G. L. 355
White, N. D. G. 753, 1494, 1497, 1851, 1921
White, P. F. 384
White, S. M. 708
Whitmore, J. 1508
Wiberg, C. 404
Wickliff, C. 1383
Wicklow, D. T. 419
Wieczorek, A. 2046
Wierbicki, E. 1069
Więsik, J. 1205
Wijsman, M. R. 2068
Wilbur, D. A., Jr. 1924
Wilken, C. 1061
Wilkes, J. 1751, 1753
Wilkin, D. R. 453, 933, 1472
Wilkin, R. 921
Wilkins, R. M. 280, 1276-1277
Willeitner, H. 674, 1185
Williams, A. C. 858
Williams, A. P. 22, 46
Williams, D. W. 1967
Williams, H. J. 1888
Williams, P. 286, 740, 1492
Williams, R. A. 130
Williams, S. T. 699
Williamson, B. 524
Willkens, G. I. 1463
Wilson, A. D. 286, 740
Wilson, C. L. 1617
Wilson, D. M. 36, 844, 1585
Wilson, J. 1508
Wilson, M. J. 256
Wilson, P. 920
Wilson, S. M. 271, 1886
Wilson, T. M. 452, 1484
Wilton-Smith, P. 407
Wimmer, F. L. 88
Winandy, J. E. 1797
Winarno, F. G. 983
Winks, R. G. 1933
Winstanley, J. K. 211
Wireman, J. W. 297, 1943-1944
Wise, D. L. 1321, 1995
Wit, J. C. de 1427
Woerth, A. 850
Wohlgemuth, R. 742, 1567
Wojciak, J. H. 1547, 1919
Wojciechowska, A. 1129
Wolf, D. 850
Woller, R. 95
Womack, H. 1587
Wong, J. 1497
Wong, J. W. 715, 1879
Woo, K. S. 214-215
Woo, S. M. 1895
Wood, B. J. B. 833
Wood, D. A. 384
Wood, D. L. 834
Wood, G. M. 443
Wood, J. M. 685
Woodburn, M. J. 392
Woolthuis, C. H. J. 551-552
Workman, M. 141
World Health Organization, Joint FAO/WHO Expert Committee on Food Safety 381
Worley, W. 1822
Wright, A. W. 1857
Wright-Rudolph, L. 1065
Wright, V. F. 1437, 1877
Wrolstad, R. E. 1636
Wu, G. X. 1569
Wu, J. F. 2002
Wyk, G. van 1784
Xie, X. Y. 657
Xu, Y. Q. 1828
Yadav, G. R. 1620
Yadav, R. S. 1883, 1898
Yadav, T. D. 959, 1563
Yamamoto, N. 1296, 1300-1303
Yang, S. S. 2017
Yang, W. Y. 1139
Yanover, S. B. 1220, 1222-1223, 1228-1229
Yasuda, K. 410
Yates, J. R. 2005
Yatome, C. 313
Yazid, M. 116
Yearsley, C. W. 1051
Yeoman, S. 2030
Yeroshchev-Shak, V. A. 814
Yin, H. F. 1920
Yonova, I. 1079
Yoon, Y. C. 587
Yordy, J. R. 1958
Yoshida, T. 1891
Yoshizawa, K. 1296
Yoshizawa, T. 459, 1722
Youdeowei, A. 1519
Young, J. C. 1560
Young, L. D. 2084
Young, W. 1819
Younus-uzzaman, M. 671-672
Yousef, A. 1364
Yousef, A. E. 191, 411
Yousef, Y. B. 1364
Yu, E. K. C. 790, 1333
Yu, K. Y. 655
Yule, R. A. 212
Yushima, T. 265
Zaadhof, K. J. 560, 573
Zabel, R. A. 643-644
Zaghloul, T. M. 832
Zagustina, N. A. 817
Zahab, D. M. 2003
Zaher, M. A. 1908
Zahora, A. R. 677, 1203
Zaika, L. 1069
Zaitsev, G. M. 780-781
Zajac, M. 1700
Zakaria, M. 832
Zalevskii, V. S. 1349
Zall, R. R. 1362
Zalom, F. G. 975
Zanina, V. V. 1228-1229
Zauberman, G. 106
Žďárková, E. 1473, 1905
Zee, J. 162
Zeiger, E. 482
Zerbini, P. E. 1605
Zerfiridis, G. K. 193, 627
Zertuche, L. 1362
Zeuthen, P. 155
Zevenhuizen, L. P. T. M. 314
Zhang, G. S. 34, 1466
Zhang, Y. Z. 2067
Zhen, Y.-Z. 7
Zherebtsov, N. A. 1387
Zhu, B. L. 897
Ziegler, H. 2026
Zik Singh 284
Zile Singh 973
Zirschky, J. 1992
Zitelli, A. 1681
Znoj, G. M. 2060
Zon, G. 874
Zong, R. 1596
Zottola, E. A. 237
Zuberi, R. 988
Zurera Cosano, G. 190
Zvyagintseva, N. P. 1220, 1223
Zwart, K. B. 2007, 2014
Zwerger, A. 244
Zylstra, G. J. 2067
Zyltman, F. 1706

SUBJECT INDEX

ımectin, control, stored products pests 1885
idia
beet pulp, utilization 1315
feeds 907
milk products, Egypt 1094
proteins, production 1315
nthoscelides obtectus
beans, deltamethrin 289
beans (*Phaseolus*)
plant extracts 738
resistance 960
control, plant extracts 738
Phaseolus coccineus 145
Phaseolus vulgaris 145
cold storage 146
fumigants 146
heat treatment 146
seed penetration 79
tricalcium phosphate 1566
rus siro
barley, control 933
biology, reproduction 1904
control, protective atmospheres 725
feeds
detection 1472
German Federal Republic 908
physical control 1473
United Kingdom 909
tic acid, beef, bacteria 1066
tivibrio cellulolyticus, cellulose, hydrolysis 346
tobacter, soft drinks, flocculation 99
tobacter aceti, vinegar 990
tobacter liquefaciens, pineapples, symptoms 1635
tobacterium woodii, lactose, biodegradation 1305
tone-butanol, production, hemicelluloses 1333
tones, production, whey 2040
tylation
timbers
Microcerotermes bresoni 1780
Poria monticola 1780
ls, production, cellulosic wastes 2016
tetobacter
4-chlorobiphenyl, biodegradation 359
metalworking fluids 1819
trichloroethylene, biodegradation 2012
waste water treatment, phosphorus, removal 2030
monium, control, ammonia 1941
lonitrile, control, *Callosobruchus chinensis* 281
dione, peaches, *Rhizopus stolonifer* 115
nidia chinensis, Botrytis cinerea, controlled atmosphere storage 1013
nidia deliciosa
Botryosphaeria dothidea 518
Botrytis cinerea 518
Sclerotinia sclerotiorum 518
vated carbon, water purification 1293
vated sludge
bacteria, toxic substances 312
beverage industry, wastes, treatment 1300
bulking
bacteria, chlorine 1978
models 1977
distillery effluents, detergents 1973
dyes, biodegradation 313
and application 1311, 1988
models 1975
nitrification 1976
phenols, biodegradation 2028
phthalic acid esters, biodegradation, *Nocardia erythropolis* 1327
sewage treatment, bacteria 1299
waste water treatment
computer program 1979
phosphorus, removal 2030
reviews 1974
Activated sludge *cont.*
yeasts, lysis 1300-1303
Adhesion, biofouling, bacteria 243
Adhesives, microbial contamination 387
Aeration, cereals, pest control 925
Aerobic digestion, waste water treatment, reviews 1980
Aeromonas
beef 162
petroleum hydrocarbons, effluents 357
shrimps, indoles 151
Aeromonas dechromatica, chromium, detoxification 1347
Aeromonas hydrophila
detection, culture media 858
food poisoning, reviews 388
Aeromonas hydrophilia, water pollution, rivers 302
Aeromonas sobria, food poisoning, reviews 388
Africa
sorghum, insect pests 1537
stored products, *Prostephanus truncatus,* reviews 1860
Agaricales, wood, reviews 651
Agaricus bisporus
cultivation, compost 321
horse manure, utilization 321
wheat straw, utilization 321
Aglossa caprealis, cereals, China 1509
Agricultural buildings
biodeterioration, USSR 839
books, USSR 839
Agricultural wastes
anaerobic treatment
Endomycopsis fibuligera 329
Trichoderma lignorum 329
artichokes
utilization 1314
fungi 1314
cabbages
utilization 1314
fungi 1314
composting 828
ethanol, production 2002
lactic acid, production 1320
reviews 2009
utilization
Lactobacillus casei 1320
Paecilomyces 2002
Agrosil, feeds, fungi 918
Ahasverus advena
cereals, Canada 58
ginger, New Zealand, interceptions 1899
stored products, Japan 1891
Air pollution, decomposition, microorganisms, soil 305
Aircraft, pest control 824
Alcaligenes
4-chlorobiphenyl, biodegradation 359
indoles, biodegradation 335
Alcaligenes faecalis
cocoa husks, utilization 1999
phenols, biodegradation 1357
Alcoholic beverages, wastes, fermentation, *Spirulina maxima* 808
Alcohols, oxidation, *Candida guilliermondii* 801
Aldrin, timbers, Isoptera 1765
Algae
building materials, organotin compounds 683
toxins, shellfish, food poisoning 1658
Algae culture, pig slurry 330
Algicides
organotin compounds 683
testing, reviews 1940
Alkylammonium compounds, wood preservatives 1800
Alloys
bacteria, seawater 1220, 1222
Bacterium halophilum, seawater 1222
Bacterium singulare, seawater 1222
Alloys *cont.*
Pseudobacterium boreale, seawater 1222
Almonds
insect pests, controlled atmospheres 1929
Solenopsis xyloni, diazinon 975
Alphitobius, distribution, Caribbean 267
Alphitobius diaperinus, stored products, Japan 1891
Alternaria
beech 652
Capsicum annuum, control 528
mycotoxins, reviews 43-44
spices 490
storage dips 1031
Alternaria alternata
apples
controlled atmosphere storage 1007
dodine 1612
Morocco 504
Planofix 1612
sugars 1611
control
ammonia 1941
plant extracts 1947
documents 1212
enzymes, cellulases 1212
fruits, physiology 106
mangoes 119
controlled atmosphere storage 1014
prochloraz 1014
Phyllanthus emblica 119
poles, strength 644
pomegranates, India 1626
sorghum, Egypt 72
Alternaria brassicae
cabbages
fungicides 536
ronilan Smoke 539
Alternaria citri
citrus fruits, wax coatings 1027
mandarins 517
Alternaria mali, apples, fungicide tolerance 300
Alternaria radicina, carrots, control 1037
Alternaria solani
foods, analysis 21
tomatoes, plastic film 527
Alternaria tenuissima, Lablab purpureus 478
Alternaria tomato, tomatoes, plastic film 527
Alteromonas putrefaciens, cod 149
Amaranthus, aflatoxins 880
Amines, foods, reviews 854
Amino acids, milk, psychrotrophic bacteria 1690
2-Aminobutane, citrus fruits, *Penicillium digitatum* 120
Amitermes evuncifer, control, repellents 1242
Ammonia, control, fungi 1941
Ammonium compounds
wood preservatives 1208, 1417, 1773
piles 1801
Ammonium propionate
hay, *Aspergillus* 1477
preservation, hay 1476
Amorphotheca resinae
fuels 1392
taxonomy 1844
Amyelois transitella
almonds, controlled atmospheres 1929
identification 262
Anacanthotermes, timbers, Saudi Arabia 1765
Anaerobic conditions, fresh water 316
Anaerobic digesters
bacteria, identification 766
cellulolytic microorganisms, enumeration 367
design 1985
meatworks effluent, Chile 362

Anaerobic digesters *cont.*
Methanobacteriaceae 767
waste water treatment 1986
Anaerobic digestion
benzene 2020
cellulosic wastes
methane production 2014
rumen microorganisms 2014
municipal refuse disposal
methane production 2007
rumen microorganisms 2007
nitrilotriacetic acid 2021
pulp mill effluent 2058
toluene 2020
waste water treatment 1984
reviews 1983
whey 765, 2052
Anaerobic fermentation
bark 315
waste waters 309
Anaerobic treatment
Methanobacteriaceae 317
septic tank effluent, filtration 1298
sewage sludge 316
sludges, Methanobacteriaceae 314
wastes, fuels, production 1321
Analysis, mycotoxins, books 844
Analytical methods, tomato concentrate, microorganisms, enumeration 130
Anastrepha ludens, mangoes, quarantine 1624
Anastrepha suspensa
grapefruits
biology 1436
temperature 1016
Animal products
feeds, *Salmonella* 1475
mycotoxins 1463
Annual reports
Agricultural Science Service, United Kingdom 1389
Australian Centre for International Agricultural Research 382
Centre for Overseas Pest Research 823
Cyprus Agricultural Research Institute 1036, 1243
India, Central Food Technological Research Institute 380
Peru , International Potato Center 1042
Anobiidae, USSR, books 1400
Anobium pertinax, control, HCN 239
Anobium punctatum
control
cypermethrin 660
HCN 239
timbers
German Democratic Republic 1769
ionizing radiation 1769
Anthonomus grandis, cotton, hydrocyanic acid 1158
Anthonomus vestitus, cotton, hydrocyanic acid 1158
Anthrenus flavipes
physiology, enzymes 1858
wool textiles
fenvalerate 679
permethrin 1816
Anthrenus verbasci
carpets, Australia, New South Wales 1866
stored products, Australia, New South Wales 1866
Antibiotics, production, whey 2046
Antifeedants, control, *Sitophilus granarius* 735
Antifoulant C9211M, control, *Trentepohlia odorata* 695
Antifouling agents
bacteria 1228
bacterial leaching 1229
Antiseptics, preservation, museum specimens 1828
Antrodia albida, physiology, temperature 1842
Antrodia carbonica, physiology, temperature 1842
Apparatus, treatment, fungicides, cabbages 1052
Apple juice
aflatoxins, potassium sorbate 895
Apple juice *cont.*
Byssochlamys fulva, ionizing radiation 494
patulin 493
determination 876
United Kingdom 993
Apples
Alternaria alternata
dodine 1612
Morocco 504
Planofix 1612
sugars 1611
Alternaria mali, fungicide tolerance 300
Archips micaceanus, malathion 503
Botryodiplodia theobromae, enzymes 127
Botryosphaeria obtusa, control 505
Botrytis cinerea, fungicides 502, 509
Ctenopseustis obliquana
chlorpyrifos 1607
controlled atmosphere storage 1608
Epiphyas postvittana
chlorpyrifos 1607
controlled atmosphere storage 1608
fungi
control 112
controlled atmosphere storage 1007
physiology 106
Panonychus ulmi, carbon dioxide 110
Penicillium expansum
calcium 114
control 505
controlled atmosphere storage 114
fungicides 502, 509
Phytophthora cryptogea, German Democratic Republic 1609
Planotortrix excessana
chlorpyrifos 1607
controlled atmosphere storage 1608
postharvest decay, control 1598
postharvest treatment, fungicides 1602
prestorage treatment
benomyl 108
calcium chloride 108
Rhizopus, control 505
storage
controlled atmosphere storage 1001-1002, 1600
diseases, France 1003
disorders 999-1000
ethoxyquin 1606
ozone 1002
quality 1005
storage decay, control 508
Venturia inaequalis, control 1004, 1603
Apricots
Plodia interpunctella, biology 1872
prestorage treatment, iodine 510
Aqua Reslin, raisins, insects 1036
Aquaculture
waste waters
power generation 1384
reviews 2006
Araecerus fasciculatus
cassava, losses 1390
coffee beans, deltamethrin 289
control, sorptive coatings 1930
Archips micaceanus, apples, malathion 503
Argentina, ice cream, *Staphylococcus aureus,* enterotoxins 1679-1680
Armillaria bulbosa, *Fagus* 649-650
Aromatic compounds
biodegradation 1323
sewage sludge, biodegradation 1355
Arsenic, leaching, *Thiobacillus ferrooxidans* 1324
Arthrobacter, frescos, biodeterioration 1226
Arthrobacter globiformis, sugar factory waste, conversion 812
Arthrographis cuboidea, timbers 232
Arthropod pests
buildings, Canada 1852
cereals, Australia 1498
millets 1542
potatoes, India 1041
sorghum 1542
Brazil 1541
stored products, Indonesia 1246
wheat, China 468
Artocarpus heterophyllus, *Rhizopus arcarpi* 1630
As compounds, wood preservatives 180
Ascorbic acid, potatoes, *Clostridium botulinum* 534
Asemum striatum, timbers, control 117
Ash, pearl millet, *Ephestia cautella* 94
Asparagus
postharvest decay, methyl bromide 1051
storage, controlled atmosphere storage 535
Aspergillus
agricultural wastes, utilization 1314
cheeses 193
control, essential oils 1952
feeds 907
USSR 911
foods 48
guavas, coatings 1627
hay, ammonium propionate 1477
milk products, Egypt 1094
mycotoxins, butter 617
olives 485
paper, foxing 233
proteins, production 1314
sorghum, Egypt 72
spices 490
taxonomy 703
books 1404
wheat 958
Aspergillus candidus
maize, morphology 1528
soyabeans, captan 475
wheat, drying 77
Aspergillus clavatus
feeds, proteases 1483
mycotoxins, reviews 1456
Aspergillus fischeri
fruit products, Czechoslovakia 997
polyvinyl chloride 1217
Aspergillus flavus
aflatoxins
control, carbon monoxide 40
groundnuts 36
maize 36, 1458
Mexico 1526
soyabeans 36
cereals, dust, sampling 867
control
ammonia 1941
essential oils 1240
plant extracts 1947, 1949
cowpeas 1578
biochemistry 1577
enzymes, tomatoes 131
genetics, aflatoxins 415
groundnuts, mustard 980
guavas, aflatoxins 1631
maize, temperature 1524
physiology, sodium chloride 706
pomegranates, India 1626
watermelons, India 1640
Aspergillus fumigatus
cellulose, biodegradation 343
control, ammonia 1941
cowpeas 1578
biochemistry 476
enzymes, tomatoes 131
malt, Nigeria 1518
polyurethanes 682
pomegranates, India 1626
single cell proteins, cellulose 340
Aspergillus glaucus
maize, storage decay 462
straw treatment 350
wheat, drying 77
***Aspergillus glaucus* group,** wheat 73
Aspergillus nidulans
cowpeas 1578
biochemistry 476
watermelons, India 1640
Aspergillus niger
bananas, control 1633
Cicer arietinum, amino acids 1576
citrus fruits 1022
control
ammonia 1941
plant extracts 1949
polyoxin 1843
cowpeas 1578

rgillus niger cont.
fruits, control 1598
maize 901
mangoes, control 516, 520
metals, extraction 1346
polyvinyl chloride 1217
pomegranates, India 1626
sulphides, oxidation 1346
whey, utilization 2043
yams, enzymes 134
rgillus ochraceus
enzymes, xylanase 2070
physiology, sodium chloride 706
xylanolytic microorganisms 2070
rgillus oryzae, cyclopiazonic acid 445
rgillus parasiticus
aflatoxins
control, homeopathic drugs 1459
groundnuts 36
maize 36
soyabeans 36
control, essential oils 1240
guavas, aflatoxins 1631
physiology, sodium chloride 706
rgillus ruber, soyabeans, captan 475
rgillus sejunctus, maize 901
rgillus terreus
cowpeas 1578
biochemistry 476
enzymes, cellulase 784
rgillus versicolor
malt, Nigeria 1518
soyabeans, captan 475
idiella hartii, yams, Ivory Coast 1647
ays
PCP, fungicidal properties 1209
polychlorinated biphenyls, biodegradation 1329
P, measurement, yeasts, estimation 22
igenus scalaris
biology 269
ecology 270
wool textiles 270
igenus unicolor
carpets, Australia, New South Wales 1866
guavas, New Zealand, interceptions 1900
physiology, enzymes 1858
stored products, Australia, New South Wales 1866
wool textiles
fenvalerate 679
permethrin 1816
apulgite
sesame
Oryzaephilus surinamensis 974
Tribolium castaneum 974
ergines, wraps 132
eobasidium pullulans
control, polyoxin 1843
orange peel, utilization 2034
windows, Finland 241
tralia
buildings, *Ctenolepisma longicaudata* 1870
carpets
Anthrenus verbasci 1866
Attagenus unicolor 1866
cereals
arthropod pests 1498
pest control 382, 750
storage 1501
Trogoderma variabile 1867
commodities, *Ctenolepisma longicaudata* 1870
Cryptolestes, migration 714
mycotoxins 446
Ommatoiulus moreletii, distribution 257
Rhyzopertha dominica, migration 714
Sitophilus oryzae, migration 714
sorghum, insect pests 1540
stored products
Anthrenus verbasci 1866
Attagenus unicolor 1866
Lasioderma serricorne 1868
Plodia interpunctella 1871
Stegobium paniceum 1868
stored products pests, resistance, insecticides 1882
Australia *cont.*
textiles
Tinea pellionella 1815
Tineola bisselliella 1815
timbers
Coptotermes acinaciformis 1764
Coptotermes frenchi 1764
insect pests, books 1402
stain fungi, reviews 1391
Tribolium castaneum, migration 714
Austria
cereals, pest control 293
timbers, insect pests, books 1403
Averrhoa carambola, storage, plastic film 1018
Avocados, fungi, physiology 106
Azaconazole, wood preservatives 1786
Azadirachta indica, potatoes, *Phthorimaea operculella* 1651
Azores, *Ommatoiulus moreletii,* distribution 257
Azospirillum brasilense
cellulolytic microorganisms 1344
nitrogen fixation
cellulose, utilization 1339
straw, utilization 1339-1340
Azospirillum lipoferum, cellulolytic microorganisms 1344
Azotobacter
petroleum hydrocarbons, effluents 357
waste water treatment
pulp mill effluent 308
sugar factory waste 308
B compounds, wood preservatives 662
Bacillus
buffalo milk, germination, heat resistance 1691
cellulase, production 788
frescos, biodeterioration 1226
milk
germination 581
India 1113
lipolysis 1112
UHT treatment 586
petroleum hydrocarbons, effluents 357
solid waste, composting 778
waste water treatment, food industry 1302
Bacillus cereus
bread, survival 1583
foods
Brazil 391
culture media 404
isolation, culture media 19
milk
hydrogen peroxide 1701
keeping quality 1714
rice, temperature 70
stainless steel, adhesion 237
susceptibility, butylated hydroxyanisole 1950
Bacillus licheniformis
leather 628
nisin, preservatives, foods 28
surfactants, oils, recovery 356
Bacillus macerans, cellulolytic microorganisms 1344
Bacillus megaterium, leather 628
Bacillus mucilaginosus, quartz, biodegradation 814
Bacillus pumilus, leather 628
Bacillus subtilis
leather 628
milk
heat treatment 1114
hydrogen peroxide 1701
packaging, decontamination 685
Bacon
Clostridium botulinum
irradiation 1069
nisin 554
preservatives 157
sugars 1069
toxins 157, 1069
Bacteria
activated sludge, bulking, chlorine 1978
anaerobes, benzene derivatives, biodegradation 358
antifouling agents 1228
beef
acetic acid 1066
Bacteria *cont.*
beef *cont.*
formic acid 1066
biodeterioration
radioactive waste 2031
uranium, mine spoil 2031
biofouling, adhesion 243
Capsicum annuum, control 528
chemical industries, pollutants, genetic engineering 328
chlorinated ethenes, biodegradation 1328
contamination, ethanol, fermentation, Brazil 97
control, veal 1072
dairy wastes, treatment 1371
digesters, pigs, manures 1322
disinfectants 757
fish, culture media 24
frescos, biodeterioration 1226
heating systems, USSR 1225
honey, Fiji 88
identification, anaerobic digesters 766
leather, proteolysis 629
metals, seawater 1220
metalworking fluids, pasteurization 1818
milk
identification 1724
infrared radiation 1705
plate count 561
ultraviolet radiation 1705
milk products, plate count 561
mine spoil, biodegradation 2061
oilfield water injection systems, biocides 1825
oils, biodegradation 798
parchment, proteolysis 629
PCP, biodegradation 2024
phenols, biodegradation 2029
pork, culture media 555
posts, Cu-Cr-As 1739
potatoes
captan 1043
carboxin 1043
thiabendazole 1043
prawns, trimethylamine oxide, reduction 540
retention, beef, carcasses 163
sewage treatment, activated sludge 1299
shellfish 147
steel, seawater 1223
sugar products, quaternary ammonium compounds 971
timbers 1740
wines, sorbic acid 991
Bacterial count
cheeses, ATP, impedance 178
cod, controlled atmosphere storage 149
cream, methodology 1682
foods, Sri Lanka 848
meat, ATP 556
milk 592, 610, 1115, 1124
ATP
determination 1137
impedance 178
Bactoscan 1142
cheeses, sensory evaluation 1715
culture media 1713
DEFT 590, 1688
German Federal Republic 1711
keeping quality 582
Korea Republic 1139
methodology 1703
methylene blue test 1140
resazurin test 1699
sterilization 1712
techniques 1720
milk products, Japan 179
pork, techniques 159
poultry meat, Japan 170
prawns
cold storage 1060
irradiation 1060
Bacterial leaching
antifouling agents 1229
metals 353
reviews 795
microorganisms, identification, genetics 2005

Bacterial leaching *cont.*
uranium, cyanobacteria 796
Bacteriophages, beef, *Pseudomonas* 1068
Bacterium halophilum, metals, seawater 1222
Bacterium singulare, metals, seawater 1222
Bacteroides cellulosolvens
cellulose, fermentation 1337
synergism, *Clostridium saccharolyticum* 1337
Bagasse
biodegradation
Cellulomonas 338
Sporotrichum pulverulentum 811
ethanol, production 324
Melanocarpus albomyces, xylanase, production 818
utilization
feeds 811
reviews 364
Bakers' yeast, production, wheat bran 806
Bamboos, preservation 1814
Banana waste
fermentation, *Candida curvata* 361
lipids, production 361
Bananas
Aspergillus niger, control 1633
Colletotrichum musae, triforine 1026
fungi, physiology 106
Bark
anaerobic fermentation 315
methane production 315
utilization, *Phanerochaete chrysosporium* 315
Bark composts, decay 764
Bark extracts
control, *Reticulitermes flavipes* 1278
fungicides 1287
insecticides 1287
Barley
Acarus siro, control 933
citrinin
estimation, liquid chromatography 431
temperature 934
fungi, Saudi Arabia 456
Fusarium 448
Lepidoglyphus destructor, control 933
ochratoxins
estimation 435
liquid chromatography 431
radioimmunoassay 460
temperature 934
preservation, *Propionibacterium rubrum* 2041
sterigmatocystin, immunoassay 444
toxinogenic fungi 461
viomellein 436
xanthomegnin 436
Basidiomycetes
cellulose, biodegradation 1731
growth, water stress 704
lignin
biodegradation 1331
estimation 351
lignocellulose, biodegradation 1731
potato waste, utilization 1313
timbers
German Democratic Republic 1743
taxonomy 1743
whey, utilization 1363
wood 646
wood preservation, testing 1193
Basilit, wood preservatives 1206
Bassia latifolia
maize, *Sitophilus oryzae* 1937
potatoes, *Phthorimaea operculella* 1651
Batocera rufomaculata, timbers, control 1766
Beans
Acanthoscelides obtectus, deltamethrin 289
mycotoxins, Yugoslavia 479
ochratoxins
Bulgaria 887
estimation 1469
Zabrotes subfasciatus
biology 1859
damage 1571
Beans (*Phaseolus*)
Acanthoscelides obtectus
plant extracts 738
resistance 960
Zabrotes subfasciatus, resistance 960
Beauveria alba
murals
p-chloro-*m*-cresol 1829
phenylmercuric acetate 1829
United Kingdom 1829
Beech, fungal decay 652
Beef
bacteria
acetic acid 1066
formic acid 1066
bacterial count 1067
ATP 556
Campylobacter jejuni, temperature 245
carcasses, contamination 163
Clostridium botulinum, toxins 32
Clostridium perfringens
cooking 1065
microwave cooking 1065
Helicostylum pulchrum, antibacterial properties 252
microbial contamination
amines 156
volatile compounds 166
microbial count 162, 544
chilling 548
cooking 1065
culture media 545
Malaysia 547
microwave cooking 1065
Mucor mucedo, antibacterial properties 252
Pseudomonas, bacteriophages 1068
Salmonella, cooling 546
Staphylococcus, Nigeria 161
Thamnidium elegans, antibacterial properties 252
Beer, preservation 1595
Beet leaf fodder, *Erysiphe betae,* safety 1490
Beet pulp
utilization
Absidia 1315
Chrysosporium 1315
Mucor 1315
Thielavia 1315
Benincasa hispida
Fusarium solani 526
Gibberella fujikuroi 526
Benomyl
apples, prestorage treatment 108
cabbages, *Botrytis cinerea* 1052
grapes 525
mangoes, prestorage treatment 116
pears, storage, diseases 1605
resistance, fungi, packhouses 507
timbers, stain fungi 1171
tomatoes, *Cladosporium oxysporum* 1644
Benzene, biodegradation, anaerobic digestion 2020
Benzene derivatives
biodegradation 358
bacteria, anaerobes 358
Benzimidazoles, bananas, *Aspergillus niger* 1633
Benzoic acid, control, yeasts 25
Beverage industry, wastes, treatment, activated sludge 1300
Beverages
Acetobacter, flocculation 99
Brettanomyces naardenensis 492
yeasts, techniques, detection 491
Bibliographies
pome fruits 111
soyabeans 86
stored products pests, control 1951
wheat 76
wood preservation 676
Bifenthrin, control, *Lasioderma serricorne* 1934
Biocides
biodegradation, testing 2022
formaldehyde 1945
hexahydro-1,3,5-triethyl-*s*-triazine 1945
military use 1291
mode of action 1943
Biocides *cont.*
oilfield water injection systems, bacteria 1825
resistance, *Pseudomonas aeruginosa* 1943
triazines, resistance 297
Biodegradation
bagasse, *Cellulomonas* 338
benzene derivatives 358
biocides, testing 2022
t-butylphenyl diphenyl phosphate 336
cellulose 791
cyanides 334
Gibberella 2010
DDT, *Phanerochaete chrysosporium* 333
2,4-dichlorophenol, *Pseudomonas* 762
dioxin, *Phanerochaete chrysosporium* 333
ferulate, *Enterobacter* 792
hemicelluloses 789
indoles 335
kinetics 304, 1958
lignin 791, 1342, 1385
estimation 351
Merulius tremellosus 341
Phanerochaete chrysosporium 376
lignocellulose 791
Lentinula edodes 1343
3-methoxy-4-hydroxycinnanate, *Enterobacter* 792
p-nitrophenol, *Pseudomonas* 762
N-nitrosodimethylamine 1330
organochlorine compounds, *Phanerochaete chrysosporium* 333
phenols, *Pseudomonas putida* 800
phthalic acid esters 1327
rice straw, India 1956
rubber, *Nocardia* 813
straw 350, 1344
tannins 332
toxic substances 1963
wood chemistry, books 841
xylan 789
Clostridium acetobutylicum 793
D-xylan 2013
Biodeterioration
agricultural buildings, USSR 839
books 1407
wood destroying fungi, reviews 1756
Biofilms
metals, *Pseudomonas atlantica* 693
waste water treatment 307
Biofouling
adhesion, bacteria 243
cooling systems 1230
marine environment, coatings 691
marine structures, control 1836
organotin compounds, paints, United Kingdom 692
Trichosporon beigelii, organic acid recovery system 1835
Biogas
production
China 1962
landfill, reviews 1995
Biological control
cereals, Lepidoptera 923
control, *Tribolium castaneum* 727
peaches, *Monilinia fructicola* 1617
stored products, mites 1905
stored products pests 1927
reviews 1925
wheat, *Sitophilus oryzae* 1550
Biological filtration, *Eichhornia crassipes,* waste water treatment 1971-1972
Biological fixed-film systems, waste water treatment, reviews 1965
Biological treatment, wastes 1966
Biomass
methane production 325
production, distillery effluents 2038
utilization, *Paecilomyces* 2002
Biomass accumulation, whey 1378
Biomineralization, books 2083
Bioresmethrin
resistance
Oryzaephilus surinamensis 279
Sitophilus oryzae 1861
Biotechnology
books 759, 1409

ɔtechnology *cont.*
cultural sociology 758
culture collections, fungi 375
dictionaries 2085
fungi, metabolites 2069
rural sociology 758
tumen, timbers, Isoptera 1765
ack pepper
insecticidal properties 1244
microflora, moisture content 1592
ɔoks
agricultural buildings, USSR 839
Anobiidae, USSR 1400
Aspergillus, taxonomy 1404
biodeterioration 1407
control 1827
fungi, India 1831
Italy 1826
biomineralization 2083
biotechnology 759, 1409, 2085
buildings, biodeterioration, United Kingdom 383
Coniophoraceae, Europe 2082
crops, diseases, control 2084
fermented foods, microbiology 833
field crops, pests, United Kingdom 836
foods, pests management 1399
fungi 846
foods 385
Sweden 1832
industrial microbiology 1409
insect pests, Cameroon 835
Lachnocladiaceae, Europe 2082
mushrooms 384
mycotoxins 843, 845
analysis 844
Canada 904
pasta, pest control 837
Penicillium
identification 386
taxonomy 1404
Polyporaceae, North America 1406
Pyralidae, stored products, United Kingdom 2080
Scolytidae, pest management 834
stored products pests 838
Canada 2081
timbers
decay fungi 840
insect pests 840
Australia 1402
Austria 1403
wood chemistry, biodegradation 841
wood preservation, Tropics 1401
ɔrax
timbers, insect pests 1766
wood preservatives 1206, 1814
oric acid
mandarins, postharvest treatments 1625
peaches, *Rhizopus stolonifer* 115
timbers, insect pests 1766
wood preservatives 1814
oron
application methods 678
timbers, *Coniophora puteana* 678
ostrichidae, timbers, Korea Republic, interceptions 214
otryodiplodia, guavas, coatings 1627
otryodiplodia theobromae
canes and rattans 1162
cassava, physiology 532
citrus fruits 1022
wax coatings 1027
fruits
enzymes 127
physiology 106
mangoes
heat treatment 1015
postharvest treatment 1622
pears, nystatin 1008
sweet potatoes
enzymes 531
physiology 532
yams
enzymes 134, 531
physiology 532
otryosphaeria dothidea, *Actinidia deliciosa* 518
otryosphaeria obtusa, apples, control 505
otrytis
Capsicum annuum, control 528
Botrytis *cont.*
leeks 144
onions 133, 144
packhouses, pome fruits 507
pears, fungicides 1605
resistance, benomyl 507
Botrytis cinerea
Actinidia chinensis, controlled atmosphere storage 1013
Actinidia deliciosa 518
apples
controlled atmosphere storage 1007
fungicides 502, 509
cabbages
benomyl 1052
carbendazim 1052
fungicides 536
testing 143
control
heat treatment 506
sodium 2-phenylphenolate 506
fruits, physiology 106
grape must, biochemistry 96
grapes 525
nectarines 1011
peaches 1011
pears 1610
raspberries 524
stone fruits 1616
South Africa 1615
strawberries, processing, wines 1636
tomatoes, ethylene 1641
Botrytis porri, leeks, Canada 1054
Botswana, wood preservation 1187
Brass
bacteria, seawater 1220, 1222
Bacterium halophilum, seawater 1222
Bacterium singulare, seawater 1222
Pseudobacterium boreale, seawater 1222
Brazil
ethanol, fermentation, bacteria, contamination 97
foods, *Bacillus cereus* 391
meat, *Salmonella* 1073
school meals, microbial flora 11
sorghum, arthropod pests 1541
Brazil nuts, aflatoxins, United Kingdom 972
Bread
Bacillus cereus, survival 1583
deoxynivalenol 414, 480
Brettanomyces naardenensis, beverages 492
Brewery effluent, reviews 2039
Bridges, decay, detection 689
Brodifacoum, rodenticides, India 274
Bromethaline, control, rats 1909
Bromophos
sesame
Oryzaephilus surinamensis 974
Tribolium castaneum 974
Bromoxynil, biodegradation, *Klebsiella pneumoniae* subsp. *ozaenae* 2025
Brucella, milk products, Mexico 1675
Bruchus pisorum, Vicia faba, New Zealand, interceptions 1899
Brussels sprouts
storage
cold storage 1047
controlled atmosphere storage 1047
Buckwheat, aflatoxins, analysis 410
Buffalo milk
Bacillus, germination, heat resistance 1691
Corynebacterium 591
microbial count 566
microbial flora 1086
Iraq 1683
sterilization 1136
preservation, lactoperoxidase system 1695-1696
storage, lactoperoxidase system 611
Building materials
algae, organotin compounds 683
insecticides, persistence 291
Building timbers
Hylotrupes bajulus, phosphine 688
preservation
alkylammonium compounds 1800
Cu compounds 1800
Building timbers *cont.*
preservation *cont.*
Cu-Cr-As 1800
wood preservation 1189
Buildings
arthropod pests, Canada 1852
biodeterioration
Italy 1823
United Kingdom, books 383
Camponotus abdominalis, USA, Florida 1265
Ctenolepisma longicaudata, Australia, New South Wales 1870
fumigation, HCN 239
fungi, control 1834
preservation
China 1833
United Kingdom 1787
Serpula lacrimans
alkalis 1227
moisture content 1227
wood preservation 666
Tanzania 1737
Bulgaria
beans, ochratoxins 887
cheeses, microbial flora 1155
ewe milk, psychrotrophic bacteria 1697
Fagus sylvatica, fungi 1741
maize, ochratoxins 887
milk, *Yersinia enterocolitica* 1132
wood preservatives 1777
Buprestis aurulenta, distribution, Poland 273
Buprestis haemorrhoidalis, distribution, Poland 273
Buprestis rustica, distribution, Poland 273
Burma, hemp, insects 1728
Burundi, foods, aflatoxins 879
Butanediol
production
lactose 1366
whey, ultrafiltration permeate 776
2,3-Butanediol
production
hemicelluloses 1333
Klebsiella pneumoniae 790
Trichoderma harzianum 790
Butanol, production, whey 1368, 2040
Butter
coliform bacteria, culture media 1671
lipolytic bacteria, culture media 1149
mycotoxins, *Aspergillus* 617
Buttermilk, *Staphylococcus aureus,* detection 397
Butylated hydroxyanisole, antibacterial properties 1950
***T*-butylphenyl diphenyl phosphate,** biodegradation, *Cunninghamella elegans* 336
Byssochlamys fulva, apple juice, ionizing radiation 494
Byssochlamys nivea, fruit products, Czechoslovakia 997
Cabbages
Alternaria brassicae
fungicides 536
ronilan Smoke 539
Botrytis cinerea
benomyl 1052
carbendazim 1052
fungicides 536
testing 143
Listeria monocytogenes
pH 1656
sodium chloride 1656
temperature 1656
Rhizopus stolonifer, fungicides 536
storage, controlled atmosphere storage 1050
Cadmium, detoxification, *Klebsiella aerogenes* 802
Cajanus cajan
Callosobruchus, India 964, 966-967
Callosobruchus chinensis, resistance 1568
Cakes, microbial flora, France 970
Calcium
apples, *Penicillium expansum* 114
peaches, storage, quality 512
Calcium chloride, apples, prestorage treatment 108

Calcium propionate
feeds
fungi 918
preservation 1478
Caliothrips fasciatus, oranges, New Zealand, interceptions 1900
Callinectes sapidus
bacterial diseases 153
Vibrio 153
Callosobruchus
Cajanus cajan, India 964, 966-967
Cicer arietinum, India 964, 966-967
flours, India 926
pulses, India 926
Vigna mungo, India 964, 966-967
Vigna radiata, India 964, 966-967
Callosobruchus analis, mung beans, neem extracts 959
Callosobruchus chinensis
cereals, plant extracts 1285
Cicer arietinum, varietal susceptibility 963
control
fumigants 281
insecticides 723
cowpeas 78
competitive ability 268
grain legumes, resistance 1568
mung beans 78
gamma radiation 474
neem extracts 922, 959
plant oils 961
Pongamia glabra 922
varietal susceptibility 80
peas 78
Phaseolus vulgaris, tricalcium phosphate 1566
Vigna angularis 78
gamma radiation 474
Vigna radiata 965
biology 962
deltamethrin 1569
piperonyl butoxide 1569
vegetable oils 1573
Callosobruchus maculatus
biology, behaviour 1894
control
dill 1288
insecticides 737
plant extracts 1863
cowpeas 78
biology 1857
reproduction 1862
control 81
deltamethrin 1569
development 266, 1570
Nigeria 82
pirimiphos-methyl 1569
resistance 1564
tricalcium phosphate 1566
varietal susceptibility 83, 969
Lablab purpureus, development 1570
mung beans 78
neem extracts 959
plant oils 961
peas 78
Phaseolus vulgaris
oviposition 721
tricalcium phosphate 1566
resistance
carbaryl 710
lindane 710
malathion 710
soyabeans, biology, development 1572
Vigna angularis 78
Vigna radiata 965
biology 962
development 1570
Vigna unguiculata, population density 968
Calotropis procera
antifeedants, *Rhyzopertha dominica* 1263
control, *Rhyzopertha dominica* 1936
Cameroon, books, insect pests 835
Camphor, timbers, Isoptera 1242
Camponotus abdominalis, buildings, USA, Florida 1265
Camponotus herculeanus, identification 261
Campylobacter
goat milk, enteritis 3
isolation, culture media 389
Campylobacter coli
meat, USA 160
pork 549
Campylobacter fetus **subsp.** ***intestinalis,*** milk, Java 183
Campylobacter jejuni
chicken meat
culture media 1075
France 851
culture media 861
eggs 1078
foods
culture media 861, 1440
reviews 856
meat
culture media 18
USA 160
milk
France 851
health hazards 1116
lactoperoxidase system 1101
pork, storage 553
survival, temperature 245
Canada
buildings, arthropod pests 1852
cereals
arthropod pests 1507
insect pests 58, 1491
feeds, deoxynivalenol 1464
food poisoning 390, 393
Salmonella typhimurium 847
foods, deoxynivalenol 1464
grain stores, pest control 753
leeks, *Botrytis porri* 1054
logs, wood borers 1180-1181
meat products, microbial count 1667
mycotoxins, books 904
stored products pests 1852
books 2081
resistance, malathion 1921
whey, utilization 2046
wood, *Phellinus tremulae* 634
wood preservation 1804
Candida, hydrocarbons, biodegradation 1351
Candida acidothermophilum, rice straw, biodegradation 1956
Candida curvata
banana waste, fermentation 361
lipids, production 361, 363
whey, fermentation 363, 1370
Candida guilliermondii, alcohols, oxidation 801
Candida pseudotropicalis
whey
fermentation 1365, 2049-2050
utilization 1364
Candida tropicalis, phenols, biodegradation 2026-2027
Candida tsukubaensis, enzymes, amylases 377
Candida utilis
coffee pulp, liquids, utilization 807
potato starch, utilization 1317
Candida wickerhamii
cellulose, hydrolysis 374
glucosidases, cellulose 374
Canes and rattans
Botryodiplodia theobromae 1162
Dinoderus, control 1178
Dinoderus minutus 1162
Heterobostrychus aequalis 1162
Penicillium 1162
stain fungi
drying, equipment 1166
PCP-Na 1166
Canned meats, safety 394
Canned products
bacteria 686
Clostridium botulinum, reviews 30
Cannery effluent, water pollution, rivers 302
Canning, equipment, bacteria 686
Canning industry, *Clostridium botulinum,* USA 247
Capsicum
storage, cold storage 1596
storage decay 1035
Capsicum annuum, storage decay, co 528
Captafol
apples, postharvest decay 505
fungicide tolerance, *Alternaria ma* 300
Litchi, Geotrichum candidum 162
tomatoes, *Cladosporium oxysporu* 1644
Captan
potatoes
bacteria 1043
Fusarium 1043
sorghum, *Sitophilus oryzae* 946
soyabeans, fungi 475
tomatoes, *Cladosporium oxysporu* 1644
Carbamates, wood preservatives 1804
Carbaryl
Ochlandra travancorica, insect pes 1729
resistance
Callosobruchus maculatus 71
Oryzaephilus surinamensis 27
Carbendazim
apples, postharvest decay 505
cabbages, *Botrytis cinerea* 1052
pears, storage, diseases 1605
wood preservatives 1804
Carbolineum, wood preservatives 674
Carbon dioxide
apples, *Panonychus ulmi* 110
cereals
Rhyzopertha dominica 1492
Sitophilus oryzae 1492
Tribolium castaneum 1492
control
Acarus siro 725
insects 740-742, 745
Rhyzopertha dominica 1254
stored products pests 295, 192
Tribolium confusum 1938
resistance, *Sitophilus oryzae* 1266
wheat, insect pests 286
Carbon disulphide
cereals, insects 748
control
insect pests 1290
Tribolium castaneum 728
wheat, insects 746
Carbon monoxide
control
aflatoxins 40
Aspergillus flavus 40
Carbon tetrachloride
control
insect pests 1290
Tribolium castaneum 728
wheat, insects 746
Carboxin
potatoes
bacteria 1043
Fusarium 1043
Cardamoms, insecticidal properties 12
Caribbean
Alphitobius, distribution 267
postharvest losses 500
Tenebrio, distribution 267
Tribolium, distribution 267
Carpets
Anthrenus verbasci, Australia, Ne South Wales 1866
Attagenus unicolor, Australia, Ne South Wales 1866
Carpophilus hemipterus
biology, temperature 1854
raisins, controlled atmospheres 19
Carrots
Alternaria radicina, control 1037
fungi, culture media 17
Casein, coliform bacteria, culture me 1671
Caseinates, *Salmonella,* culture medi 1082
Cashews, *Suidasia pontifica,* New Ze land, interceptions 1900
Cassava
aflatoxins 424
Araecerus fasciculatus, losses 13
Botryodiplodia theobromae, physi 532

ava *cont.*
asioderma serricorne, losses 1390
rostephanus truncatus
control 1892
Kenya 1892
Tanzania 530, 1892
ngle cell proteins, *Rhizopus chinensis,* poultry manure 331
orage decay, metabolites 1649
ava meal, ochratoxins, estimation 1469
ia nigricans, insecticidal properties 738
r, maize, *Sitophilus oryzae* 1937
r oil, control, *Callosobruchus maculatus* 81
e manure, fermentation, cellulolytic microorganisms 779
obacter, cooling systems, fouling 1230
y
riomyza trifolii, cold storage 1053
orage, controlled atmosphere storage 1050
biose, *Cellulomonas* 337
lases
llulose digestion, enzymes 1336
search, reviews 339
richoderma reesei, mutants 819
lolytic microorganisms
cetivibrio cellulolyticus 346
spergillus fumigatus, single cell proteins 340
zospirillum brasilense 1344
zospirillum lipoferum 1344
acillus 788
acillus macerans 1344
ittle manure, fermentation 779
ellulomonas 337
ellulomonas fermentans 344
ellulomonas gelida 1344
ellvibrio 368
lavispora 794
lostridium acetobutylicum 345
lostridium thermocellum 352, 1386, 1736
mposts, Kuwait 348
orynascus sepedonium 1237
umeration, anaerobic digesters 367
eotrichum candidum, enzymes 817
chnoderma resinosum 653
olation, culture media 303
lebsiella pneumoniae 790
kes, USSR 787
falbranchea pulchella 1237, 1239
fyceliophthora thermophila 1387
enicillium expansum 1849
leurotus sajor-caju 372
olyplastron multivesiculatum 1160
clerotium rolfsii, enzymes 782
hielavia 1237
richoderma harzianum 790
richoderma lignorum 1237
richoderma reesei, enzyme activity 349
richoderma viride, culture media 816
ulomonas
agasse, biodegradation 338
llobiose 337
llulolytic microorganisms 337
llulose, biodegradation 1339
raw, biodegradation 1339-1340
ylanase, activity 338
ulomonas fermentans
llulolytic microorganisms 344
llulose digestion 344
ulomonas flavigena, cocoa husks, utilization 1999
ulomonas gelida
llulolytic microorganisms 1344
raw, biodegradation 1340
ulomonas uda
nzymes 1338
cellulases, genetic engineering 2065-2066
ylan, biodegradation 1338
lose
odegradation 791, 841
Aspergillus terreus 784
basidiomycetes 1731
Cellulomonas 1339
Coriolus versicolor 631
fungi 343

Cellulose *cont.*
biodegradation *cont.*
Klebsiella pneumoniae 790
Phanerochaete chrysosporium 631
Phellinus pini 631
Polyplastron multivesiculatum 1160
Pycnoporus sanguineus 631
Stereum frustulosum 631
Trichoderma harzianum 790
Clostridium thermocellum, morphology 1736
Coriolus versicolor, enzymes 1735
fermentation
Bacteroides cellulosolvens 1337
Clostridium saccharolyticum 1337
fungi, electron microscopy 198
hydrolysis, *Candida wickerhamii* 374
Trichoderma reesei, models 1335
utilization, nitrogen fixation, *Azospirillum brasilense* 1339
Cellulose digestion, enzymes, cellulases 1336
Cellulosic wastes
acids, production 2016
anaerobic digestion
methane production 2014
rumen microorganisms 2014
biodegradation
Clostridium thermocellum 783
Clostridium thermohydrosulfuricum 783
ethanol, production 783, 2018
fermentation, *Trichoderma album* 2017
fuels, production 2016
microbial treatment 1358
proteins, production 2017
sugars, production 2018
utilization
Pachysolen tannophilus 2018
Trichoderma viride 2018
Cellvibrio, cellulolytic microorganisms 368
Cephitinea colonella, cereals, China 1509
Ceraceomyces sublaevis, flax 630
Cerambycidae
biology 1249
timbers, India 1249
Ceramic tile adhesives, microbial contamination, standards 387
Ceratitis capitata
control
fenthion 1253
trichlorfon 1253
fruits, ethylene dibromide 996
lemons, resistance 117
pawpaws
ethylene dibromide 1623
quarantine 1623
peaches, heat treatment 1010
Ceratocystis fagacearum, Quercus, methyl bromide 1774
Ceratocystis paradoxa, pineapples 124
Ceratocystis pilifera, control, polyoxin 1843
Cercospora solanicola, potatoes, India 1652
Cereals
aflatoxins, Ethiopia 929
arthropod pests
Australia 1498
Canada 1507
Callosobruchus chinensis, plant extracts 1285
Cryptolestes ferrugineus, monitoring, traps 1497
Cryptolestes pusillus, monitoring, traps 1437
deoxynivalenol 458
detection 930, 1471, 1515
estimation 45, 881
gas chromatography 432
Japan 459
United Kingdom 434
dust, *Aspergillus flavus,* sampling 867
fumigants, movements, reviews 754
fumigation, reviews 1500
fungi 932
Fusarium
culture media 1444
German Federal Republic 917

Cereals *cont.*
insect pests
Canada 58, 1491
books 2081
China 1509
cold resistance 752
control 453
detection, staining 928
infrared radiation 1505
Israel 1506
microwave radiation 1505
monitoring 1506
Poland 454
resistance 1502
reviews 1502
spread 6
traps 714
United Kingdom 453
vacuums 1505
insecticides, losses 751
insects
carbon disulphide 748
integrated control 749
phosphine 748
Laemophloeus, India 926
Lepidoptera, biological control 923
mycotoxins
China 1461
Korea Republic 890, 1513
Nigeria 1546
South Africa 875
Taiwan 1461
United Kingdom 1514
USSR 1461
nivalenol
detection 930
estimation 881
Japan 459
patulin
moisture content 931
temperature 931
pest control
aeration 925
Australia 382, 750
Austria 293
ethyl formate 744
integrated control 288
storage structures 12
United Kingdom 921
Plodia interpunctella, microbial pesticides 1493
Rhyzopertha dominica
carbon dioxide 1492
heating 1504
India 926
methyl bromide 1492
Sitophilus oryzae
carbon dioxide 1492
control 285
India 926
methyl bromide 1492
moisture content 928
plant extracts 1285
Sitotroga cerealella, India 926
storage
Australia 1501
earth-covered bunkers 1503
South East Asia 1495
USA, Idaho 1508
stored products pests
insecticides 1511
monitoring, traps 1437
toxinogenic fungi 883
Tribolium castaneum
carbon dioxide 1492
India 926
methyl bromide 1492
Trogoderma granarium, plant extracts 1285
Trogoderma variabile, Australia, New South Wales 1867
wastes, treatment, reviews 2037
Cerrena unicolor, timbers, wood chemistry 200
Chaetomium, spices 490
Chaetomium cellulolyticum
proteins, production 1319
pulp mill effluent, utilization 1381
straw, upgrading 1319
Chaetomium globosum
beech 652

Chaetomium globosum *cont.*
control, polyoxin 1843
particleboards, adhesives 1202
poles, strength 644
polyurethanes 682
straw, biodegradation 342
timbers
logging, seasonal variation 640
resistance 645
Chaetomium thermophile, cellulose, biodegradation 343
Chalcophora mariana, distribution, Poland 273
Chalk, wheat, *Sitotroga cerealella* 1283
Cheese milk, *Clostridium tyrobutyricum,* determination 1717
Cheese spread, *Clostridium botulinum,* toxins 29
Cheeses
aflatoxins 626-627, 1096
analysis 410
Italy 571
Aspergillus 193
bacterial count, ATP, impedance 178
Brucella, Mexico 1675
Clostridium tyrobutyricum 1092
coliform bacteria
control 621
culture media 620, 1671
temperature 620
Enterobacteriaceae 619, 1154
Egypt 1095
temperature 622
Escherichia coli, detection 195
fungi, culture media 1151
Gram negative bacteria, Limulus test 565
keeping quality 194
Lactobacillus buchneri
health hazards 1152
histamine 1152
microbial flora 568-569, 1153
Bulgaria 1155
cold storage 624
Iraq 1676
sorbic acid 1157
Micrococcus, identification 1726
mycotoxins 192
Penicillium 193
Penicillium roquefortii 192
1,3-pentadiene 1150
Salmonella
German Federal Republic 1684
reviews 1684
Salmonella typhimurium 847
food poisoning 847, 1156, 1425
Italy 1425
Staphylococcus, identification 1726
Staphylococcus aureus, enterotoxins 1727
sterigmatocystin 1686
estimation 1725
Streptococcus 623
culture media 618
Yersinia enterocolitica
acidity 625
temperature 625
Chemical industries
effluents, biodegradation, cyanobacteria 820
pollutants, bacteria, genetic engineering 328
waste water treatment, reviews 2056
Cherries, prestorage treatment, iodine 510
Cheyletus eruditus, feeds, German Federal Republic 908
Chhana
microbial flora 1086
packaging 1089
Chicken meat
aflatoxins 1079
bacterial count, ATP 556
Campylobacter jejuni
culture media 1075
France 851
temperature 245
microbial count, Malaysia 547
Salmonella
culture media 171
decontamination 558
Chicken meat *cont.*
Salmonella typhimurium, polyphosphates 1076
Chile
garlic, *Penicillium corymbiferum* 1650
meatworks effluent, anaerobic digesters 362
China
biogas, production 1962
buildings, preservation 1833
cereals
insect pests 1509
mycotoxins 1461
feeds, mycotoxins 1461
foods, mycotoxins 1461
methane production 1962
oranges, storage 1619
stored products, mites 1907
structural wood, *Reticulitermes largus* 1247
toxinogenic fungi, foods 7
wheat, arthropod pests 468
Chinese cabbages
Phytophthora porri 537
storage
cold storage 1047
controlled atmosphere storage 1047-1049
Chlordane, timbers, Isoptera 1765
Chlordimeform, control, *Tribolium castaneum* 290
Chlorella, marble, biodeterioration 1823
Chlorinated anilines, biodegradation, *Pseudomonas diminuta* 1325-1326
Chlorinated ethenes, biodegradation, bacteria 1328
Chlorinated hydrocarbons
timbers
fungi 1810
Gloeophyllum trabeum 1793
wood preservatives 1793, 1810
Chlorine
activated sludge, bulking, bacteria 1978
chicken meat, *Salmonella* 558
citrus fruits, storage decay 521
Chlorine dioxide, citrus fruits, storage decay 521
***P*-chloro-*m*-cresol,** murals, *Beauveria alba* 1829
2-Chlorobenzoic acid, utilization, *Pseudomonas ceracia* 780
4-Chlorobenzoic acid
utilization
Corynebacterium sepedonicum 781
Pseudomonas putida 781
4-Chlorobiphenyl
biodegradation
Acinetobacter, genetic engineering 359
Alcaligenes, genetic engineering 359
Chlorobromomethane, control, *Trogoderma granarium* 271
2-Chlorophenol, sewage sludge, biodegradation 1355
3-Chlorophenol, sewage sludge, biodegradation 1355
4-Chlorophenol, sewage sludge, biodegradation 1355
Chloropicrin
preservation, poles 1808
timbers
Ganoderma applanatum 223
Poria carbonica 223
wood preservatives 223
Chlorothalonil, fungicide tolerance, *Alternaria mali* 300
Chlorpyrifos
apples
Ctenopseustis obliquana 1607
Epiphyas postvittana 1607
Planotortrix excessana 1607
control
Ephestia cautella 1284
Lasioderma serricorne 1934
Chlorpyrifos-methyl
maize
insect pests 1521
Prostephanus truncatus 936
resistance, *Oryzaephilus surinamensis* 279
Chlorpyrifos-methyl *cont.*
wheat
Oryzaephilus surinamensis 921
Sitophilus granarius 921
Tribolium castaneum 921
Chondrostereum purpureum, *Fagus* 648
Chromium, detoxification, *Aeromonas dechromatica* 1347
Chrysosporium
beet pulp, utilization 1315
proteins, production 1315
Cicer arietinum
Aspergillus niger, amino acids 1576
Callosobruchus, India 964, 966-967
Callosobruchus chinensis
resistance 1568
varietal susceptibility 963
Cinnamomum cassia, control, stored products pests 1863
Citric acid
potatoes, *Clostridium botulinum* 53
production, whey 2043
Citrobacter
milk 610
milk products, Egypt 1095
Citrus
wastes
fermentation
Corynebacterium glutamicum 1359
Propionibacterium shermanii 1360
glutamic acid, production 1359
treatment, *Rhodopseudomonas* 1295
vitamin B12, production 1360
Citrus fruits
Alternaria citri, wax coatings 1027
Aspergillus niger 1022
Botryodiplodia theobromae 1022
wax coatings 1027
Fusarium, wax coatings 1027
Penicillium digitatum 1022
control 120
wax coatings 1027
Penicillium italicum 1022
wax coatings 1027
storage, tropics 1012
storage decay, control 521
Cladosporium
feeds 907
milk products, Egypt 1094
polyvinyl chloride 1217
spices 490
Cladosporium cladosporioides
control, polyoxin 1843
paints
Nigeria 1821
walls 1821
Cladosporium herbarum
beech 652
documents 1212
enzymes, cellulases 1212
plant residues, utilization 2000
Cladosporium oxysporum
paints
Nigeria 1821
walls 1821
tomatoes, control 1644
Cladosporium resinae, taxonomy 1844
Cladosporium sphaerospermum
paints
Nigeria 1821
walls 1821
Clams, bacteria 147
Clavispora, D-xylose, fermentation 794
Clitocybe aurantiaca, growth, water stress 704
Clostridium
milk 1092
silage 1092
Clostridium acetobutylicum
cellulase, activity 345
hemicelluloses, utilization 1333
whey
fermentation 1374-1375
utilization 2040
xylan, biodegradation 793
Clostridium beyerinckii, whey, utilization 1368

Clostridium botulinum
bacon
irradiation 1069
nisin 554
preservatives 157
sugars 1069
toxins 157, 1069
beef, toxins 32
canned products, reviews 30
canning industry, USA 247
cheese spread, toxins 29
control, phosphates 1839
fish
potassium chloride 541
sodium chloride 541
inhibition
citric acid 1838
sodium acid pyrophosphate 27
meat products, phosphates, reviews 1668
potatoes, preservatives 534
spore germination, inhibition 406
toxins
detection 31
immunoassay 407
pH 873
water content 873
Clostridium perfringens
culture media 278
foods, culture media 1433, 1442
meat products
cooking 1065
microwave cooking 1065
salmon, food poisoning 1661
shellfish, United Kingdom 542
spores, germination 248
susceptibility, butylated hydroxyanisole 1950
Clostridium saccharolyticum
cellulose, fermentation 1337
synergism, *Bacteroides cellulosolvens* 1337
Clostridium thermocellum
cellulase 352
cellulolytic microorganisms 352, 1386, 1736
cellulose, morphology 1736
cellulosic wastes, biodegradation 783
enzymes 1386
Clostridium thermohydrosulfuricum, cellulosic wastes, biodegradation 783
Clostridium tyrobutyricum
cheeses 1092
milk
culture media 577, 1105
determination 1717
Coatings
biofouling, marine environment 691
guavas, postharvest decay 1627
Coccinia indica, *Colletotrichum destructivum* 123
Cochliobolus lunatus, plant residues, utilization 2000
Cocoa
Corcyra cephalonica, Ghana 984
Ephestia cautella, Ghana 984
Cocoa beans
storage
humidity 983
temperature 983
Cocoa husks
single cell proteins, production 1999
utilization
Alcaligenes faecalis 1999
Cellulomonas flavigena 1999
Cod
bacterial count, controlled atmosphere storage 149
Campylobacter jejuni, temperature 245
Coffee
aflatoxins 487-488
estimation 982
Hypothenemus hampei, Jamaica 1589
toxinogenic fungi, Japan 486
Coffee beans, *Araecerus fasciculatus,* deltamethrin 289
Coffee pulp, liquids, utilization, *Candida utilis* 807
Coke, industrial wastes, reviews 2063
Coke industry, waste waters, germanium, recovery 2062
Cold air, control, *Tribolium confusum* 1938
Cold storage
Capsicum, storage 1596
celery, *Liriomyza trifolii* 1053
cheeses, microbial flora 624
cucumbers, storage 1596
ewe milk, psychrotrophic bacteria 1698
milk
microbial flora 587
psychrotrophic bacteria 588, 1694
pears, *Penicillium expansum* 1604
Phaseolus vulgaris
Acanthoscelides obtectus 146
storage 1596
prawns, bacterial count 1060
Coleoptera
cereals, Canada 1507
control
abamectin 1885
fenoxycarb 1919
stored products, India 1252
timbers 1182
yams, Ivory Coast 1647
Coliform bacteria
cheeses
control 621
culture media 620
temperature 620
dried milk 595
foods
culture media 859
enumeration 864
estimation, reviews 405
infant formulae 595
milk 1120
Poland 1129
milk products 560
culture media 1671
poultry meat, enumeration 863
Coliform count, milk 592, 1124
Colletotrichum, guavas, coatings 1627
Colletotrichum coccodes
foods, analysis 21
potatoes, controlled atmosphere storage 1046
Colletotrichum destructivum, *Coccinia indica* 123
Colletotrichum musae, bananas, triforine 1026
Collops hirtellus, attractants, wood preservatives 708
Commodities, *Ctenolepisma longicaudata,* Australia, New South Wales 1870
Compositae, antifeedants, stored products pests 731
Composting
agricultural wastes 828
bark, decay 764
municipal refuse disposal, Kuwait 348
municipal wastes
sewage sludge, temperature 763
USA 1967
refuse, Pakistan 326
sewage sludge
aeration 1968
design 1970
temperature 1297, 1968
USA 1969
solid waste
Bacillus 778
thermophilic bacteria 777
Composts
Kuwait, cellulolytic microorganisms 348
land application 1311, 1988
sewage sludge, *Salmonella* 760
Computer program, activated sludge, waste water treatment 1979
Condensed milk, *Streptococcus,* heat resistance 564
Confectionery
microbial contamination 89
microbial flora
France 970
India 1090
Conferences
Advancing agricultural production in Africa, CAB 1
American Wood-Preservers' Association 1192-1198, 1397
Conferences *cont.*
Anaerobic waste water treatment 1305, 1395
Aspects of pest control in the food industry 824, 1258-1260, 1281, 1396
Biotechnology for fuels and chemicals 2047-2048, 2073
Canadian Pest Management Society 752-753, 825, 1161
Composting of agricultural and other wastes 828
Durability of building materials and components 827
Environmental physiology of plant disease 13
Eureopean Congress of Entomologists 1251
Fifth Meeting on Mycotoxins in Animal & Human Health 434-437, 439-444, 450-451, 831
Food hygiene 559, 573, 618-619, 2079
Food hygiene and food quality 560, 574, 620
Food industries and the environment 2045-2046, 2072
Foodborne microorganisms and their toxins 2075
Forest products research international — achievements and the future 1782-1789, 2078
Interlab 1717, 2074
International Creosote Conference 661
International on phytopharmacy and phytiatry 509, 539
International sorghum entomology workshop 1537-1542
International symposium on plant protection in tropical agriculture 1571
International working conference on stored-product entomology 1430, 1437, 1472, 1499-1509, 1521, 1543, 1554-1557, 1566-1568, 1587, 1590-1591, 1730, 1873-1892, 1906-1907, 1917-1931, 2076
Italian National Congress of Entomology 1855
Microbial associations in food 859-860, 1122-1124, 1394
Microbial ecology 830
Microorganisms and food 849-851, 870, 907, 970, 1117-1120, 1153, 1393
Mycotoxin symposia, Third International Mycological Congress, Tokyo 829
National Seminar on Behavioural and Physiological Approaches in the Management of Crop Pests, India 922, 1250, 1279
New Zealand Weed and Pest Control Conference 1607-1608
Pacific Regional Wood Anatomy Conference 201
Pacific timber engineering conference 1189-1190, 1833
Postharvest handling of vegetables 1005, 1038, 1049-1050
Practical aspects of controlled atmosphere and fumigation in grain storages 719-720, 740-750
Quality of vegetables 103
Rattan seminar 1162, 1166, 1178
Recent Advances in Rodent Control 832
The role of biochemistry in food and energy production 1649, 1995, 2015-2018
Tropical Region, American Society for Horticultural Science 1012-1013
VI international conference on the mycoses 1462-1464, 1527
Wood preservation — research and practice 826
Workshop on biotechnology in forest science 1398
Workshop on wood protection 1768, 1779, 2077
World Conference on Animal Production 399

Coniophora puteana
control
F compounds 675
wood preservatives 1208, 1777
fibreboards, F compounds 1201
particleboards
adhesives 1202
F compounds 1749
furcarbanil 1749
timbers
boron 678
F compounds 1790
logging, seasonal variation 640
petroleum oils 1200
resistance 645
wood properties 203
Coniophoraceae, Europe, books 2082
Contact lens formulations, preservatives, evaluation 1219
Controlled atmosphere storage
Actinidia chinensis, Botrytis cinerea 1013
apples
Ctenopseustis obliquana 1608
Epiphyas postvittana 1608
fungi 1007
Penicillium expansum 114
Planotortrix excessana 1608
storage 1600
cod, bacterial count 149
fruits, microbial contamination 107
groundnuts, microflora 1585
mangoes, fungi 1014
pears, *Penicillium expansum* 1604
potatoes, storage decay 1046
shrimps 152
storage decay, reviews 13
tomatoes 1641
vegetables, microbial contamination 107
Controlled atmospheres
almonds, insect pests 1929
control, stored products pests 1928
raisins, insect pests 1929
wheat, *Sitophilus granarius* 1556
Cooling
cereals, *Sitophilus oryzae* 285
tobacco, *Lasioderma serricorne* 1591
Cooling systems, fouling, bacteria 1230
Copper, tomatoes, *Cladosporium oxysporum* 1644
Copper oxychloride
ginger, fungi 1593
tomatoes, *Cladosporium oxysporum* 1644
Copper-8-quinolinolate, wood preservatives 1812
Copper sulphate, fungicides, thatch 690
Coptotermes acinaciformis
timbers
Australia 1764
New Zealand 1764
Coptotermes formosanus
timbers
consumption 1767
resistance 657
Coptotermes frenchi
timbers
Australia 1764
New Zealand 1764
Corcyra cephalonica
biology, development 1877
cocoa, Ghana 984
control
gamma radiation 1289
growth regulators 729
neem extracts 1274
sterile insect release 1926
lentils, development 1563
millets, moisture content 455
rice
moisture content 455
reproduction 1853
sorghum
ecology 1544
moisture content 455
reproduction 1853
Coriandrum sativum
Lasioderma serricorne 94
Trogoderma granarium 94
Coriolus, timbers, wood chemistry 1172
Coriolus hirsutus, timbers, resistance 647
Coriolus versicolor
cellulose
biodegradation 631
enzymes 1735
control, PCP 1209
enzymes, cellulase 1735
Fagus 648, 650
ligninolytic microorganisms 1841
particleboards
adhesives 1202
electron microscopy 201
F compounds 1749
furcarbanil 1749
plywood, laboratory tests 639
poles, physiology 1752
timbers 1209
durability 1168
hydrogen peroxide 1175
preservation 1812
resistance 645, 647, 1744
wood chemistry 1747
wood properties 203
Corynascus sepedonium
cellulolytic microorganisms 1237
enzymes, cellulases 1237
Corynebacterium
buffalo milk 591
cooling systems, fouling 1230
Corynebacterium glutamicum, citrus, wastes, fermentation 1359
Corynebacterium sepedonicum
4-chlorobenzoic acid, utilization 781
2,4-dichlorobenzoic acid, utilization 781
Coryphaena hippurus
spoilage, indexes 154
urocanic acid, production 154
Cottage cheese
keeping quality 194
manufacture, milk quality 174
microbial flora, sorbic acid 1157
Cotton
Anthonomus grandis, hydrocyanic acid 1158
Anthonomus vestitus, hydrocyanic acid 1158
bacteria, adhesion 1817
fungi, electron microscopy 198
Pectinophora gossypiella, control, machinery 1730
pest control
fumigation 1158
India 1158
Sacadodes pyralis, hydrocyanic acid 1158
Cottonseed
aflatoxins 41
detection 1450
Ephestia cautella, India 973
Cottonseed oilmeal, aflatoxins, decontamination 482
Coumarins, rodenticides 275
Cowpea meal
aflatoxins
temperature 477
water content 477
Cowpeas
aflatoxins
temperature 477
water content 477
Aspergillus flavus, biochemistry 1577
Aspergillus fumigatus, biochemistry 476
Aspergillus nidulans, biochemistry 476
Aspergillus terreus, biochemistry 476
Callosobruchus chinensis 78
competitive ability 268
resistance 1568
Callosobruchus maculatus 78
biology 1857
reproduction 1862
control 81
deltamethrin 1569
development 266, 1570
Nigeria 82
pirimiphos-methyl 1569
resistance 1564
tricalcium phosphate 1566
varietal susceptibility 83, 969
insect pests, reviews 1565
Cowpeas *cont.*
storage decay 1578
Cr-As-F, wood preservatives 1795
Cr compounds, wood preservatives 1807
Cr-F, wood preservatives 1795
Crab meat, microbial flora 148
Cream
aflatoxins 1096
bacterial count, methodology 1682
Brucella, Mexico 1675
keeping quality 563
plate count 576
microbial flora 563
Iraq 1676, 1683
psychrotrophic bacteria, sensory evaluation 1677
Salmonella
German Federal Republic 1684
reviews 1684
Creosotes
poles, preservation 1784
timbers
Lentinus lepideus 1750
Perenniporia tephropora 1750
Pycnoporus coccineus 1750
wood preservatives 220, 227-228, 661, 671-672, 674, 1412, 1419-1422, 1773, 1776, 1784, 1788, 1795, 1802, 1912
Criocephalus rusticus, timbers, control 1179
Crop residues
artichokes
utilization 1314
fungi 1314
cabbages
utilization 1314
fungi 1314
utilization, *Paecilomyces* 2002
Crops
diseases, control, handbooks 2084
losses, pest control, tropics 1
Cryptococcus, xylan, biodegradation 2019
Cryptococcus albidus, polygalacturonase, production 253
Cryptolestes
control, fumigants 748
migration, Australia 714
physiology, aggregation pheromones 1879
rice, control 66
Cryptolestes ferrugineus
cereals
Canada 58, 1491, 1507
cold resistance 752
monitoring, traps 1497
control, methyl chloroform 736
oats, methoprene 1516
rapeseed 481
resistance
malathion 1851
Canada 1921
phosphine 272, 292
rye
malathion 1494
pirimiphos-methyl 1494
sorghum, insecticides 1567
traps, aggregation pheromones 715
triticale
malathion 1494
pirimiphos-methyl 1494
wheat
cypermethrin 1548
fenvalerate 1548
malathion 1494
pirimiphos-methyl 1494
refrigeration 1554
Cryptolestes pusillus
cereals, monitoring, traps 1437
control, fumigants 746
lentils, development 1563
wheat, sorptive silica dusts 1558
Cryptophagidae, cereals, Canada 1491
Cryptotermes cynocephalus, timbers, resistance 1771
Cryptotermes domesticus, structural wood, India 654
Cryptotermes dudleyi, structural wood, India 654

Ctenolepisma longicaudata
buildings, Australia, New South Wales 1870
commodities, Australia, New South Wales 1870
Ctenopseustis obliquana
apples
chlorpyrifos 1607
controlled atmosphere storage 1608
Cu-As, wood preservatives 1417
Cu-As-F-phenol, wood preservatives, piles 1801
Cu-B-F, wood preservatives 637
Cu compounds, wood preservatives 662, 1191, 1800
Cu-Cr, wood preservatives 1795, 1912
Cu-Cr-As
posts
bacteria 1739
decay 1210
timbers, *Lecythophora mutabilis* 1759
wood preservatives 662, 665, 669, 1184, 1191, 1210, 1413, 1759, 1773, 1776, 1795, 1798-1800, 1811
Cu-Cr-B, wood preservatives 1795
Cu-Cr-B-F, wood preservatives 1208
Cu-Cr-F, wood preservatives 1185
Cucumbers
Dacus cucurbitae
methyl bromide 1638
quarantine 1638
Dacus dorsalis
methyl bromide 1638
quarantine 1638
keeping quality
packing 1032
storage 1032
Mycosphaerella, handling, techniques 1030
storage
cold storage 1596
packing 1033
temperature 1033
Culinary herbs
microflora 489
quality, USA 989
Cultural products
Salmonella
German Federal Republic 1684
reviews 1684
Cultural sociology, biotechnology 758
Culture collection catalogues
India 1405
India, Defence Materials Stores Research Development Establishment 842
National Collection of Industrial Bacteria, United Kingdom 1408
New Zealand 1392
USA 1410
Culture collections, fungi, biotechnology 375
Culture media
Aeromonas hydrophila, detection 858
Bacillus cereus, isolation 19
bacteria, fish 24
beef, microbial count 545
Campylobacter, isolation 389
Campylobacter jejuni 861
foods 861
meat 18
cellulolytic microorganisms, isolation 303
cereals, *Fusarium* 1444
cheeses, fungi 1151
Clostridium perfringens 278
foods
Bacillus cereus 404
Campylobacter jejuni 1440
Clostridium perfringens 1433, 1442
coliform bacteria 859
Enterobacteriaceae 1438
fungi 868
microbial flora 399
Salmonella 1441
Shigella sonnei 1443
Staphylococcus aureus 1435, 1445
Yersinia 857
fungi, foods 17
lactobacilli, foods 23
Culture media *cont.*
milk
bacterial count 1713
Escherichia coli 1133
Shigella sonnei 1133
Yersinia 1709
milk products, coliform bacteria 1671
pork, bacteria 555
Pseudomonas 398
Pseudomonas aeruginosa 4
psychrotrophic bacteria 398
Salmonella
detection 401
isolation 14, 20
Staphylococcus aureus, isolation, meat 1071
yeasts, foods 23
Cultured milks
microbial flora
Iraq 1672
Morocco 1085
Salmonella, survival 176
Cultured products, quality 1081
Cunninghamella elegans
t-butylphenyl diphenyl phosphate, biodegradation 336
1-nitropyrene, detoxification 355
Cuprammonium caprylate, wood preservatives 1811
Curd, *Staphylococcus aureus,* detection 397
Curvularia ovoideae, watermelons, India 1640
Curvularia verruculosa, pineapples 124
Cyanides
biodegradation
Gibberella 2010
Rhizopus oryzae 334
industrial wastes, treatment, reviews 2032
Cyanobacteria
chemical industries, effluents, biodegradation 820
genetic engineering 821
uranium, bacterial leaching 796
Cyfluthrin
control, *Prostephanus truncatus* 1939
maize, *Sitophilus zeamais* 935
Cylindrocarpon, potatoes, controlled atmosphere storage 1046
Cynaeus angustus, identification 1875
Cypermethrin
control
insect pests, flour mills 1243
Lasioderma serricorne 291, 1934
maize, *Sitophilus zeamais* 60
Ochlandra travancorica, insect pests 1729
raisins, insects 1036
resistance, *Sitophilus oryzae* 1861
wheat
Cryptolestes ferrugineus 1548
Tribolium castaneum 1548
wood preservatives 660
Cyprus
flour mills
Ephestia 1243
Plodia interpunctella 1243
Tribolium 1243
raisins
Ephestia 1036
Plodia interpunctella 1036
Cytophaga, cooling systems, fouling 1230
Cytospora pini, timbers, wood cement 638
Czechoslovakia
fruit products
Aspergillus fischeri 997
Byssochlamys nivea 997
Talaromyces flavus 997
2,4-D
biodegradation, dissolved oxygen 2023
sewage sludge, biodegradation 1355
Dacus cucurbitae
cucumbers
methyl bromide 1638
quarantine 1638
fruits
quarantine 1597
refrigeration 1597
pawpaws
ethylene dibromide 1623
Dacus cucurbitae *cont.*
pawpaws *cont.*
quarantine 1623
vegetables
quarantine 1597
refrigeration 1597
Dacus dorsalis
cucumbers
methyl bromide 1638
quarantine 1638
fruits
quarantine 1597
refrigeration 1597
mangoes, ethylene dibromide 1020
pawpaws
ethylene dibromide 1623
quarantine 1623
vegetables
quarantine 1597
refrigeration 1597
Dacus tryoni, mangoes, dimethoate 118
Dacus xanthodes, melons, New Zealand, interceptions 1899
Dahi
Enterobacter aerogenes 567
Escherichia coli 567
microbial flora 570
Dairy factories
cooling water, psychrotrophic bacteria 238
pasteurizers, psychrotrophic bacteria 189
Dairy products
Clostridium botulinum, toxins 29
microbial contamination 89
microbial flora, Iraq 181
Dairy wastes
lactic acid, production 1320
reviews 2054
treatment, bacteria 1371
utilization, *Lactobacillus casei* 1320
Dates
Ephestia cautella, gamma radiation 1645
preservation, potassium sorbate 1021
DDT
biodegradation
Phanerochaete chrysosporium 333
Pseudomonas aeruginosa 1356
resistance
Oryzaephilus surinamensis 279
Sitophilus oryzae 1902
sorghum, *Sitophilus oryzae* 946
Dead wood, decay, wood moisture 1167
Debarking, timbers, insect pests 1766
Decay fungi
control, HCN 239
physiology 249
temperature 1842
poles, strength 644
timbers
books 840
logging, seasonal variation 640
wood
wood anatomy 641
wood chemistry 641
Decayed wood, utilization 634
DEF, control, *Tribolium castaneum* 290
DEFT, milk, bacterial count 590, 1688
Delonix regia
control, *Tribolium castaneum* 1898
sterilization, *Tribolium castaneum* 1883
Deltamethrin
beans, *Acanthoscelides obtectus* 289
cereals, *Sitophilus oryzae* 285
coffee beans, *Araecerus fasciculatus* 289
control
insect pests 737
Lasioderma serricorne 291
Prostephanus truncatus 1939
cowpeas, *Callosobruchus maculatus* 1569
dried fish
Dermestes maculatus 1056
Necrobia rufipes 1056
maize
Prostephanus truncatus 936
Sitophilus zeamais 60, 289
resistance, *Sitophilus oryzae* 1861

Deltamethrin *cont.*
sorghum, insect pests 71
Vigna radiata, Callosobruchus chinensis 1569
Demeton-S-methyl, control, *Callosobruchus chinensis* 723
Dendryphiella salina, beech 652
Denitrification, oxidation ditches 1981
Denitrifying microorganisms, *Thiosphaera pantotropha* 768, 803
Denmark
stored products pests 2071
wheat, ochratoxins 957
Dermestes maculatus
dried fish
deltamethrin 1056
pirimiphos-methyl 1056
tetrachlorvinphos 1056
physiology, enzymes 1858
Dermestidae, cereals, Canada 1491
Desulfotomaculum nigrificans, heating systems, USSR 1225
Desulfovibrio desulfuricans
steel 1221
soil 1224
Detergents
biodegradation, *Pseudomonas* 799
distillery effluents, activated sludge 1973
Diacetyl, control, toxinogenic fungi 1452
Diazinon, control, *Callosobruchus chinensis* 723
2,4-Dichlorobenzoic acid
utilization
Corynebacterium sepedonicum 781
Pseudomonas putida 781
1,2-Dichloroethylene, biodegradation, bacteria 1328
Dichlorophen
analysis, wood preservatives 231
spectrophotometry 231
2,4-Dichlorophenol, biodegradation, *Pseudomonas* 762
Dichlorvos
control
insect pests, flour mills 1243
Lasioderma sericorne 1934
grain stores, insects 1271
mills
Ephestia cautella 1272
Tribolium castaneum 1272
raisins, insects 1036
resistance
Oryzaephilus surinamensis 279
stored products pests, Australia 1882
rice, *Sitotroga cerealella* 1286
timbers
Isoptera 1242
wood borers 1179
tobacco, *Ephestia elutella* 985
wheat
Rhyzopertha dominica 1553
Sitophilus oryzae 1553
Tribolium castaneum 1553
wood preservatives 659
Dicloran
nectarines, postharvest decay 1011
peaches, postharvest decay 1011
Dictionaries, biotechnology 2085
Dieldrin, timbers, Isoptera 1765
Diflubenzuron, control, insects 1275
Digesters, pigs, manures, bacteria 1322
Dill, control, stored products pests 1288
Dimethoate
control, *Callosobruchus chinensis* 723
mangoes, *Dacus tryoni* 118
Dinitrobutylphenol, wood preservatives 1184
Dinoderus, canes and rattans, control 1178
Dinoderus minutus
canes and rattans 1162
Ochlandra travancorica, control 1729
Dinoderus ocellaris, *Ochlandra travancorica,* control 1729
Dioxin, biodegradation, *Phanerochaete chrysosporium* 333
Diphenylamine, grapes 525
Disinfectants
bacteria 757
fungi 757
Disinfectants *cont.*
Pseudomonas aeruginosa 299
Distillery effluents
activated sludge, detergents 1973
biomass, production 2038
utilization, *Spirulina maxima* 2038
Dithianon, apples, postharvest decay 505
Dithiocyanatomethane, preservation, museum specimens 1828
DNA hybridization, enterotoxins, detection 874
Documents
Alternaria alternata 1212
Cladosporium herbarum 1212
Pleospora herbarum 1212
Dodine, apples, *Alternaria alternata* 1612
Dothiorella dominicana, mangoes, Nigeria 1629
DPX H6573
apples
Botrytis cinerea 509
Penicillium expansum 509
Dried fish
Dermestes maculatus
deltamethrin 1056
pirimiphos-methyl 1056
tetrachlorvinphos 1056
Necrobia rufipes
deltamethrin 1056
pirimiphos-methyl 1056
tetrachlorvinphos 1056
Dried foods, microbial count, techniques, reconstitution 402
Dried fruit
insect pests, India 853
pest control, ethyl formate 744
Plodia interpunctella, biology 1872
Dried milk
aflatoxins
estimation 614
immunoassay 1723
Italy 1143
United Kingdom 1147
coliform bacteria 595
fungi, Egypt 1094
Salmonella
German Federal Republic 1684
reviews 1684
Staphylococcus aureus
detection 184
enumeration, culture media 594
yeasts, Egypt 1094
Dried plants, *Idaea bonifata* 687
Dried skim milk
coliform bacteria, culture media 1671
fungi, Egypt 1094
Salmonella
culture media 1082
detection 572
Streptococcus, heat resistance 564
yeasts, Egypt 1094
Dried tubers, pest control, ethyl formate 744
Drinking yoghurt, endotoxins, Limulus test 1678
Drosophila melanogaster
control, electric field 294
raisins, controlled atmospheres 1929
Drug formulations, microbial contamination, Poland 235-236
Drugs, precursors, sugar factory waste 812
Drying
equipment, canes and rattans, stain fungi 1166
wheat
Aspergillus candidus 77
Aspergillus glaucus 77
Dyes, biodegradation, activated sludge 313
Economics, ethanol, production, whey 2044
Edible fungi, production, whey 2046
Edifenphos, control, rice, discoloration 1535
Education, postharvest systems, India 1423
Effluents, chemical industries, biodegradation, cyanobacteria 820
Egeria densa, waste water treatment 378
Eggs
Campylobacter jejuni 1078
ochratoxins 1080
Egypt
foods, mites 1268
Egypt *cont.*
infant formulae, microbial flora 180
milk
psychrotrophic bacteria 1127
thermoduric bacteria 1127
milk products
Enterobacteriaceae 1095
fungi 1094
yeasts 1094
potatoes, postharvest decay 142
poultry meat, microbial contamination 168
sorghum, fungi 72
stored products, mites 1908
vegetables, microbial count 538
Eichhornia crassipes
biological filtration, waste water treatment 1971-1972
waste water treatment 378-379
Electric field, *Drosophila melanogaster,* control 294
Eleocharis dulcis, storage 1038
ELISA, meat, ochratoxins 1064
Embelin, wheat, *Tribolium castaneum* 470
Endomycopsis fibuligera, agricultural wastes, anaerobic treatment 329
Endosulfan
timbers
insect pests 658
wood borers 1179
wood preservatives 659
Endotoxins
milk, Limulus test 1678
milk products, Limulus test 1678
Enterobacter
ferulate, biodegradation 792
3-methoxy-4-hydroxycinnanate, biodegradation 792
milk products, Egypt 1095
phosphorus, release 804
Enterobacter aerogenes
dahi 567
milk, hydrogen peroxide 1701
Enterobacter cloacae, whey, utilization 1366
Enterobacteriaceae
cheeses 619, 1154
temperature 622
foods, culture media 1438
identification 15
milk 592
milk products 560
Egypt 1095
isolation 177
sewage, USA 761
Enterotoxins
Escherichia coli
detection 408, 874, 1447
immunoassays 872
milk products 559
Staphylococcus
detection, immunoassays 1449
foods 1446
meat 1446
Staphylococcus aureus
cheeses 1727
foods, detection, thermonuclease 1448
ice cream 871
Argentina 1679-1680
milk products 175
Yersinia enterocolitica, milk 870
Enterovirus, isolation, sludges 301
Enumeration, cellulolytic microorganisms, anaerobic digesters 367
Environmental pollution, microorganisms, inoculation 762
Enzyme activity, *Trichoderma reesei,* cellulose digestion 349
Enzymes
amylases, *Candida tsukubaensis* 377
cellulose digestion, cellulases 1336
immobilization, *Saccharomyces anamensis* 370
production, whey 1361
Ephestia
control, insecticides 1243
flour mills, Cyprus 1243
raisins
Cyprus 1036
insecticides 1036

phestia cont.
raisins *cont.*
microbial pesticides 1036
stored products, Japan 1891
traps 263
warehouses, United Kingdom 1889
phestia cautella
cocoa, Ghana 984
control
fumigants 747
gamma radiation 1932
growth regulators 729
insecticides 1284
methacrifos 1264
phosphine 1256
pirimiphos-methyl 1264
protective atmospheres 741
sterile insect release 1926
traps 1889
2-tridecanone 1865
cottonseed, India 973
dates, gamma radiation 1645
flours, India 926
groundnuts
ecology 1586
growth regulators 976
pheromones 976
lentils, development 1563
maize, population dynamics 1896
military rations 855
mills, dichlorvos 1272
pearl millet, control 943
population dynamics 1873
pulses, India 926
resistance
insecticides, Australia 1882
lindane 1864
malathion 1864, 1897
sorghum, control 71
Ephestia elutella
control
sterile insect release 1926
traps 1889
tobacco, dichlorvos 985
Ephestia figulilella
control, sterile insect release 1926
raisins, controlled atmospheres 1929
Ephestia kuehniella
cereals, Poland 454
control
methyl chloroform 736
protective atmospheres 741
sterile insect release 1926
traps 1889
feeds, German Federal Republic 908
figs, New Zealand, interceptions 1899
physiology, enzymes 1858
resistance, insecticides, Australia 1882
wheat
heat treatment 466
proteins 467
Turkey 471
Epicoccum nigrum, taxonomy 1844
Epicoccum purpurascens, taxonomy 1844
Epiphyas postvittana
apples
chlorpyrifos 1607
controlled atmosphere storage 1608
Erwinia, phosphorus, release 804
Erwinia carotovora, tomatoes, plastic film 527
Erwinia carotovora* subsp. *atroseptica
potatoes 141
varietal reactions 1654
Erwinia carotovora* subsp. *carotovora
onions 136
potatoes
enzymes 1653
grading 1044
Erwinia chrysanthemi
enzymes, pectinases 1236
mutants 698
Erysiphe betae, beet leaf fodder, safety 1490
Escherichia, phosphorus, release 804
Escherichia coli
beef
acetic acid 1066
formic acid 1066
cheeses 1153
detection 195
Escherichia coli cont.
dahi 567
detection 1840
enterotoxins
detection 408, 874, 1447
immunoassays 872
milk products 559
food poisoning 1425
foods
detection 862
enumeration 864
milk 592, 1124
culture media 1133
formaldehyde 1692
hydrogen peroxide 1701
inhibition 1110
reactivation 1099
volatile compounds 1689
milk products 560
Egypt 1095
poultry meat
Egypt 168
enumeration 863
shellfish, United Kingdom 542
susceptibility, butylated hydroxyanisole 1950
textiles, adhesion 1817
Essential oils, control, fungi 1240, 1952
Etaconazole
apples
Botrytis cinerea 509
Penicillium expansum 509
Ethanol
fermentation, bacteria, contamination, Brazil 97
production
agricultural wastes 2002
bagasse 324
cellulosic wastes 783, 2018
lignocellulose, reviews 2015
whey 1362, 1365, 1367, 1372, 2042, 2045-2048, 2050
economics 2044
xylan 2019
xylose 2068
D-xylose 2003
Ethiopia, cereals, aflatoxins 929
3-Ethoxycarbonyl oxyl-1-bromo-1,2-diiod-propene, wood preservatives 1812
Ethoxyquin, apples, storage, disorders 1606
Ethyl formate
control
Callosobruchus chinensis 281
insects 744
Ethylene
tomatoes
Botrytis cinerea 1641
Penicillium 1641
Ethylene dibromide
control
Callosobruchus chinensis 281
insect pests 1290
Tribolium castaneum 728
fruits, *Ceratitis capitata* 996
mangoes, *Dacus dorsalis* 1020
pawpaws
Ceratitis capitata 1623
Dacus cucurbitae 1623
Dacus dorsalis 1623
Ethylene dichloride
control
insect pests 1290
Trogoderma granarium 271
Ethylene oxide, control, insect pests 1290
Etrimfos
barley
Acarus siro 933
Lepidoglyphus destructor 933
cereals, stored products pests 1511
control
Ephestia cautella 1284
insect pests 737, 1282
maize, *Sitophilus zeamais* 60
pearl millet, *Trogoderma granarium* 1536
rice, *Sitotroga cerealella* 1286
sesame
Oryzaephilus surinamensis 974
Tribolium castaneum 974
sorghum
Cryptolestes ferrugineus 1567
Etrimfos *cont.*
sorghum *cont.*
Oryzaephilus surinamensis 1567
Rhyzopertha dominica 1567
Tribolium castaneum 1567
Trogoderma granarium 1567
wheat
Oryzaephilus surinamensis 921
Sitophilus granarius 921
Tribolium castaneum 921
Eucalyptus
decay, preservation 637
Trametes lilacino-gilva 1753
wood anatomy 1751
Eupatoriopicrin, antifeedants, stored products pests 730
Europe
Coniophoraceae, books 2082
Lachnocladiaceae, books 2082
wood preservation, organizations 1183
Euryomma peregrinum, hides, New Zealand 196
Euzopherodes vapidella, yams, Ivory Coast 1647
Euzophora bigella, peaches, United Kingdom 1614
Ewe milk
microbial flora, Iraq 1672
psychrotrophic bacteria
Bulgaria 1697
cold storage 1698
storage, methylene blue test 608
F compounds
fibreboards, *Coniophora puteana* 1201
particleboards
Coniophora puteana 1749
Coriolus versicolor 1749
Poria placenta 1749
timbers, *Coniophora puteana* 1790
wood preservatives 675, 1201, 1749, 1790
piles 1801
Fabric, bacteria, adhesion 1817
Fagus, fungi, ecology 648-650
Fagus sylvatica, fungi, Bulgaria 1741
Farinocystis tribolii, control, *Tribolium castaneum* 727
Feed grains, toxinogenic fungi, India 1480
Feed mills, insect pests, Canada, books 2081
Feeds
aflatoxins 447, 449, 892, 912
detection 916, 1481
ELISA 437
liquid chromatography 426
estimation 1482
moisture content 42
Spain 1467
temperature 42
Turkey 915
Venezuela 913-914
animal products, *Salmonella* 1475
arthropod pests, German Federal Republic 908
Aspergillus clavatus, proteases 1483
bagasse, utilization 811
citrinin
detection 897
estimation 450
deoxynivalenol
Canada 1464
USA 1464
diacetoxyscirpenol, bioassays 1486
fungi 907
Agrosil 918
calcium propionate 918
USSR 911
fusarins 1484
Fusarium 1474
German Federal Republic 917
Gibberella fujikuroi 452
mites
detection 1472
determination 400
enumeration 1269
physical control 1473
United Kingdom 909
moniliformin 1484
mycotoxins 56, 420, 452, 1451, 1474
China 1461
Finland 39

Feeds *cont.*
mycotoxins *cont.*
German Federal Republic 910
Hungary 1487-1488
reviews 50
South Africa 875
Taiwan 1461
United Kingdom 451
USSR 1461
ochratoxins 919
detection 897
estimation 435, 450
Penicillium 420
preservation 1479
calcium propionate 1478
production
microorganisms 773
molasses 2033
straw 1318
whey 2033, 2043
Salmonella, detection 1434, 1489
sterigmatocystin, estimation 905
sugarcane pith, digestion 322
T-2 toxin, bioassays 1486
trichothecenes, estimation 1460
waste utilization 329
zearalenone 425
ELISA 417
Fenitrothion
control
Callosobruchus chinensis 723
Ephestia cautella 1284
insect pests 737
maize, *Sitophilus zeamais* 935
Ochlandra travancorica, insect pests 1729
resistance
insects 719
Oryzaephilus surinamensis 279, 287
stored products pests, Australia 1882
rice, *Sitotroga cerealella* 1286
sorghum
Cryptolestes ferrugineus 1567
insect pests 71
Oryzaephilus surinamensis 1567
Rhyzopertha dominica 1567
Tribolium castaneum 1567
Trogoderma granarium 1567
timbers, wood borers 1179
wheat
Oryzaephilus surinamensis 921
Sitophilus granarius 921
Tribolium castaneum 921
wood preservatives 659
Fenobucarb, timbers, insect pests 658
Fenoxycarb
control, stored products pests 1919
wheat, insect pests 1547
Fenthion
control
Callosobruchus chinensis 723
Ceratitis capitata 1253
Fenvalerate
control, *Lasioderma serricorne* 291
maize
Prostephanus truncatus 936
Sitophilus zeamais 60
resistance, *Sitophilus oryzae* 1861
sorghum, insect pests 71
timbers, Isoptera 1765
wheat
Cryptolestes ferrugineus 1548
Tribolium castaneum 1548
wool textiles
Anthrenus flavipes 679
Attagenus unicolor 679
Tineola bisselliella 679
Fermentation wastes, reviews 2039
Fermented foods
microbial contamination, storage 1594
microbiology, books 833
Staphylococcus aureus, detection 397
Ferulate, biodegradation, *Enterobacter* 792
Fibreboards
Coniophora puteana, F compounds 1201
wood preservation, methodology 226
Field crops, books, pests, United Kingdom 836
Figs, *Ephestia kuehniella*, New Zealand, interceptions 1899
Fiji
honey, bacteria 88
timbers, stain fungi 667
wood preservation 666
Filtration
bacteria, odour abatement 311
septic tank effluent, anaerobic treatment 1298
Finland
feeds, mycotoxins 39
windows, fungi 241
Fish
bacteria, culture media 24
bacterial count, controlled atmosphere storage 149
Clostridium botulinum
potassium chloride 541
sodium chloride 541
histamine, estimation 1057
microbial count, ice 150
Polyplastron multivesiculatum 1058
spoilage, indexes 154
urocanic acid, production 154
Vibrio parahaemolyticus, smoking 1660
Fish scrap, treatment, reviews 2036
Fishponds, waste waters, microorganisms 1306
Flavobacterium
cod 149
cooling systems, fouling 1230
marble, biodeterioration 1823
meat 165
milk 1119
organotin compounds, leaching 1229
PCP, biodegradation 2024
shrimps, indoles 151
Flax
Ceraceomyces sublaevis 630
maize, *Sitophilus oryzae* 1937
Flocoumafen
control
rats 1910
rodents 1911
Floors, preservation 673
Flour mills
Ephestia, Cyprus 1243
insect pests, Canada, books 2081
Plodia interpunctella, Cyprus 1243
Tribolium, Cyprus 1243
Flours
Callosobruchus, India 926
Ephestia cautella, India 926
fungi, culture media 17
Pyralis farinalis, biology 1855
Flucythrinate
maize
Sitophilus zeamais 60-61
Sitotroga cerealella 61
Folpet, fungicide tolerance, *Alternaria mali* 300
Food industry
environmental protection, conferences 2072
pest control
conferences 1396
reviews 1281
Psocoptera, United Kingdom 1258
waste water treatment
Bacillus 1302
Oerskovia 1302
Streptomyces 1302
Food poisoning
Aeromonas hydrophila, reviews 388
Aeromonas sobria, reviews 388
Brazil 391
Canada 390, 393
cheeses, *Salmonella typhimurium* 847, 1156, 1425
economics 1424
Escherichia coli 1425
low fat milk, *Salmonella typhimurium* 1710
milk, *Salmonella typhimurium* 596-597
salmon, *Clostridium perfringens* 1661
Salmonella 1425
Salmonella typhimurium, Canada 847
shellfish, algae, toxins 1658
Food poisoning *cont.*
Staphylococcus aureus, Iran 1426
Yersinia enterocolitica 1425
Food products
Clostridium botulinum, preservatives 534
Staphylococcus aureus, Nigeria 10
Foods
aflatoxins 46, 892
Burundi 879
chemistry 886
detection, liquid chromatography 426
ELISA 38
Indonesia 902
Japan 902
Philippines 902
Spain 1467
USSR 438
amines, reviews 854
Bacillus cereus
Brazil 391
culture media 404
bacterial count, Sri Lanka 848
Campylobacter jejuni
culture media 861, 1440
reviews 856
citrinin 46
Clostridium perfringens, culture media 1433, 1442
coliform bacteria
culture media 859
enumeration 864
estimation, reviews 405
deoxynivalenol
Canada 1464
USA 1464
Enterobacteriaceae, culture media 143
Escherichia coli
detection 862
enumeration 864
food hygiene, inspection, United Kingdom 395
fungi
analysis 21
books 385
culture media 17, 868
mycotoxins 416
hygiene, conferences 2079
insect pests 855
New Zealand 852
lactobacilli, culture media 23
microbial contamination 1427
estimation 860
immunological techniques 1439
microbial count, safety 392
microbial flora
conferences 1393-1394
culture media 399
Morocco 849
techniques 16
microorganisms, toxins, conferences 2075
mites, Egypt 1268
mycotoxins 420, 1451
China 1461
Fusarium 413
German Federal Republic 884
India 1465
Japan 418, 421, 888
legislation, USA 2
reviews 50
Taiwan 1461
USSR 1461
patulin 46, 49
Penicillium 420
pest control, conferences 824
pests management, books 1399
preservation, irradiation, New Zealand 1428
production, microorganisms 773
Psocoptera, United Kingdom 1259-1260
psychrotrophic bacteria, enzymes, proteases 696
Ptinus tectus, New Zealand, interceptions 1899
safety, reports 381
Salmonella
culture media 1441
detection 403, 1432, 1434

Foods *cont.*
Salmonella cont.
estimation, reviews 405
immunoassay 869
immunological techniques 396
Shigella sonnei, culture media 1443
Staphylococcus, enterotoxins, Netherlands 1446
Staphylococcus aureus
antibiotics, resistance 246
culture media 1435, 1445
enterotoxins, detection, thermonuclease 1448
Iran 1426
toxinogenic fungi 48
bioassays 443
China 7
trichothecenes, estimation 1460
yeasts, culture media 23
Yersinia 850
culture media 857
France 857
Netherlands 1431
Yersinia enterocolitica
detection 1429
France 857
Forecasting, insect pests, sex attractant traps 265
Formaldehyde
biocides 1945
biodegradation, *Pseudomonas fluorescens* 1354
milk
Escherichia coli 1692
Salmonella typhimurium 1692
Staphylococcus aureus 1692
Streptococcus lactis 1692
Streptococcus thermophilus 1692
resistance
Pseudomonas 1945
Pseudomonas aeruginosa 1943-1944
Pseudomonas putida, genetics 1946
Formic acid, beef, bacteria 1066
Formica, attractants, wood preservatives 708
Formicidae, irrigation equipment, USA, Hawaii 1824
Foxing, paper, *Aspergillus* 233
France
apples, storage, diseases 1003
cakes, microbial flora 970
chicken meat, *Campylobacter jejuni* 851
foods
Yersinia 857
Yersinia enterocolitica 857
milk
Campylobacter jejuni 851
Yersinia enterocolitica 1706
pears, storage, diseases 1003
Frescos, biodeterioration, bacteria 1226
Fresh water, anaerobic conditions 316
Fruit juices
Acetobacter, flocculation 99
Leuconostoc oenos 101
microbial flora, Nigeria 102
patulin 49
Fruit products
Aspergillus fischeri, Czechoslovakia 997
Byssochlamys nivea, Czechoslovakia 997
Talaromyces flavus, Czechoslovakia 997
Fruits
Ceratitis capitata, ethylene dibromide 996
Dacus cucurbitae
quarantine 1597
refrigeration 1597
Dacus dorsalis
quarantine 1597
refrigeration 1597
fungi
India 1599
USSR 998
insect pests
irradiation 496
New Zealand 852, 995
quarantine certification, USA 497
Fruits *cont.*
microbial contamination, controlled atmosphere storage 107
patulin 49
postharvest losses, Caribbean 500
postharvest storage decay, reviews 126
postharvest systems, reviews 495
storage 380
wraps, reviews 104
wastes, treatment, reviews 2037
Fuels
Amorphotheca resinae 1392
production
cellulosic wastes 2016
wastes, anaerobic treatment 1321
whey 1321
Fumigants
control, *Sitophilus granarius* 1273
maize, insects 747
modes of action, reviews 1290
movements, cereals, reviews 754
Phaseolus vulgaris, Acanthoscelides obtectus 146
phosphine 743
resistance, stored products pests 1922
timbers, preservation 1796
wood preservatives 1796
Fumigation
cabbages, *Alternaria brassicae* 539
cotton, pest control 1158
Fungal decay, timbers, reviews 1757
Fungal protein, production, *Gibberella zeae* 1998
Fungi
apples
control 112
controlled atmosphere storage 1007
biotechnology, metabolites 2069
books 846
biodeterioration, India 1831
Sweden 1832
buildings, control 1834
cellulose
biodegradation 343
electron microscopy 198
cereals 932
Saudi Arabia 456
cheeses, culture media 1151
control, sorbic acid 1920
culinary herbs 989
culture collections, biotechnology 375
detection, culture media 277
disinfectants 757
Fagus sylvatica, Bulgaria 1741
feeds 907
Agrosil 918
calcium propionate 918
USSR 911
foods
analysis 21
books 385
culture media 17, 868
mycotoxins 416
fruits
India 1599
USSR 998
groundnuts, packaging materials 93
growth, materials testing 5
guavas, India 1628
hay 1485
homes, Sweden 240
lignin, biodegradation 641
mangoes, gamma radiation 1618
milk 589
infrared radiation 1705
ultraviolet radiation 1705
milk products, Egypt 1094
paintings, India 1830
palm kernel oil, fatty acids 981
paper
organotin compounds 756
testing, standards 1211
particleboards 1176
pawpaws, gamma radiation 1618
pears, control 112
physiology 249
poles
methyl isothiocyanate 1203
USA 643
polymers 1215
polyvinyl chloride 1217
Fungi *cont.*
Psophocarpus tetragonolobus, control 1580
rice 942
silage 589
sorghum 948
Egypt 72
soyabeans 87
heating 1579
spices 989
straw 1485
biodegradation 342
sunflower seeds 1584
timbers 1161
control 199
detection, spectrometry 1745
methyl bromide 224
oxygen requirement 1762
preservation 1810
wood preservation 205
tomato products, estimation 1643
vegetables
India 1599
Saudi Arabia 105
USSR 998
Vigna aconitifolia 84
Vigna radiata 85
windows, Finland 241
wood chips 207
storage 1170
wood preservation, laboratory tests 1912
Fungicide tolerance
apples, *Alternaria mali* 300
pome fruits, *Penicillium expansum* 113
Fungicides
apples
Botrytis cinerea 502, 509
Penicillium expansum 502, 509
cabbages
Alternaria brassicae 536
Botrytis cinerea 536
Rhizopus stolonifer 536
copper sulphate, thatch 690
lemons, *Penicillium digitatum* 1621
organotin compounds 756
paints, testing 1822
pears
Botrytis 1605
Penicillium 1605
tangors, *Penicillium digitatum* 1621
tomatoes, postharvest treatment 129
wood preservatives, testing 1418
Fungus control, wood, tributyltin oxide 230
Furcarbanil
particleboards
Coniophora puteana 1749
Coriolus versicolor 1749
Poria placenta 1749
wood preservatives 1749
Furniture
Anobium punctatum
German Democratic Republic 1769
ionizing radiation 1769
Dinoderus, control 1178
Fusarium
cereals
culture media 1444
German Federal Republic 917
citrus fruits, wax coatings 1027
Fagus 648
feeds 1474
USSR 911
guavas, coatings 1627
maize 1474
mangoes
controlled atmosphere storage 1014
prochloraz 1014
mycotoxins
foods 413
moniliformin 1454
polyvinyl chloride 1217
potatoes
captan 1043
carboxin 1043
controlled atmosphere storage 1046
thiabendazole 1043
spices 490
storage dips 1031
wheat 440
Poland 472

Fusarium avenaceum, feed grains 448
Fusarium culmorum
feed grains 448
potatoes, controlled atmosphere storage 1046
Fusarium equiseti, ginger, control 1593
Fusarium oxysporum
foods, analysis 21
onions 136
potatoes, epidemiology 1045
Fusarium poae, feed grains 448
Fusarium sambucinum* var. *ossicola, taxonomy 1844
Fusarium solani
Benincasa hispida 526
guavas, India 1632
Lablab purpureus 478
maize 901
onions 136
Fusarium sporotrichioides, trichothecenes, wheat 899
Fusarium sulphureum, potatoes, controlled atmosphere storage 1046
Fusarium tricinctum, mycotoxins 896
Galium, lead, effluents, absorption 360
Gallionella, cooling systems, fouling 1230
Gamma radiation
control
Corcyra cephalonica 1289
Ephestia cautella 1932
Tribolium castaneum 713, 727-728, 1255
Tribolium confusum 1938
dates, *Ephestia cautella* 1645
groundnuts, aflatoxins 1588
maize, *Sitophilus zeamais* 59
mangoes, fungi 1618
mung beans, *Callosobruchus chinensis* 474
pawpaws, fungi 1618
pest control
fruits 496
vegetables 496
sorghum, *Sitophilus zeamais* 59
Vigna angularis, Callosobruchus chinensis 474
Ganoderma applanatum
sawdust, anatomy 635
timbers
chloropicrin 223
wood chemistry 200, 1169
Tsuga heterophylla, anatomy 635
Ganoderma lucidum, timbers, decay 1760
Ganoderma tsugae, timbers, decay 1760
Garlic, *Penicillium corymbiferum,* Chile 1650
Gas production, landfill 771
Genetic engineering
Acinetobacter, biodegradation, 4-chlorobiphenyl 359
Alcaligenes, biodegradation, 4-chlorobiphenyl 359
Cellulomonas uda, cellulases 2065-2066
chemical industries, pollutants, bacteria 328
cyanobacteria 821
whey, utilization, *Saccharomyces cerevisiae* 2053
Geotrichum candidum
cellulolytic microorganisms, enzymes 817
citrus fruits, control 521
control
potassium sorbate 522
sodium 2-phenylphenolate 522
sorbic acid 522
foods, analysis 21
Lablab purpureus, India 1657
lemons
potassium sorbate 522
sodium 2-phenylphenolate 522
Litchi
captafol 1620
nickel chloride 1620
nickel sulphate 1620
milk products, morphology 1235
taxonomy 1844
tomatoes, plastic film 527
watermelons, India 1640
German Democratic Republic
apples, *Phytophthora cryptogea* 1609
timbers
Anobium punctatum 1769
basidiomycetes 1743
German Federal Republic
cereals, *Fusarium* 917
feeds
arthropod pests 908
mycotoxins 910
foods, mycotoxins 884
milk
bacterial count 1711
legislation 598
milk products, *Salmonella* 1684
wood preservation, standards 670
wood preservatives, standards 1204
Germanium
recovery
coke industry, waste waters 2062
Pseudomonas putida 2004, 2062
Ghana
cocoa
Corcyra cephalonica 984
Ephestia cautella 984
Gibberella
cyanides, biodegradation 2010
mycotoxins, foods 413
Gibberella avenacea, potatoes, controlled atmosphere storage 1046
Gibberella fujikuroi
Benincasa hispida 526
cowpeas 1578
feeds 452
onions 136
yams, enzymes 134
Gibberella pulicaris, potatoes, controlled atmosphere storage 1046
Gibberella zeae
feed grains 448
fungal protein, production 1998
maize, survival 1525
mycotoxins 896
Gibbium aequinoctiale, stored products, distribution 1257
Gibbium psylloides, stored products, distribution 1257
Ginger
aflatoxins, detection 1450
Ahasverus advena, New Zealand, interceptions 1899
Fusarium equiseti, control 1593
Phytosciara zingiberis, Japan 987
Psilosciara flammulinae, Japan 987
Pythium pleroticum, control 1593
Gliocladium roseum
fruits, control 1598
polyurethanes 682
Gliocladium virens, taxonomy 1844
Gloeophyllum saepiarium
poles, physiology 1752
timbers 633
Gloeophyllum trabeum
control, plant extracts 1287
physiology 249
plywood, laboratory tests 639
poles, physiology 1752
timbers
detection 1165
durability 1168
preservation 1793
resistance 647, 1744
Gloeosporium, guavas, coatings 1627
Glomerella, guavas, coatings 1627
Glomerella cingulata
fruits, physiology 106
mangoes
heat treatment 1015
postharvest treatment 1622
pomegranates, India 1626
Glucosidases, cellulose, *Candida wickerhamii* 374
Glutamic acid, production, citrus, wastes 1359
Glycyphagus domesticus, feeds, German Federal Republic 908
Gnathotrichus retusus, logs, Canada 1180
Gnathotrichus sulcatus, logs, Canada 1180-1181
Goat milk
aflatoxins, metabolism 412
Goat milk *cont.*
Campylobacter, enteritis 3
microbial flora 602
milk quality 578
pasteurization 1106
United Kingdom 186
milkborne diseases 603-604
storage, methylene blue test 608
Grain elevators, insect pests, Canada, books 2081
Grain stores
insects, United Kingdom 920
pest control
Canada 753
insecticides 1271
stored products pests, historical recor 1510
Gram negative bacteria
cheeses, Limulus test 565
milk 606, 609
culture media 1721
Limulus test 565
Grape juice
patulin 100
trichothecin 100
detection 992
Grape must, *Botrytis cinerea,* biochemistr 96
Grapefruits
Anastrepha suspensa
biology 1436
temperature 1016
Penicillium digitatum, ethylene 121
storage, controlled atmosphere storage 513
Grapes
benomyl 525
Botrytis cinerea 525
diphenylamine 525
prestorage treatment, iodine 510
storage
growth regulators 1637
sulphur dioxide 1029
Graphium rigidum, control, polyoxin 1843
Greece
ice cream, microbial flora 1084
milk, microbial flora 605
Groundnut butter, aflatoxins, United Kingdom 972
Groundnuts
aflatoxins 36
analysis 410
detection 979, 1450
gamma radiation 1588
hygroscopicity 92
Singapore 37
storage 978
United Kingdom 972
Aspergillus flavus, mustard 980
Ephestia cautella
ecology 1586
growth regulators 976
pheromones 976
fungi, packaging materials 93
insect pests, USA, Georgia 1587
microflora, controlled atmosphere storage 1585
mycotoxins 483
ochratoxins, estimation 1469
Plodia interpunctella, ecology 1586
storage decay, plant extracts 1949
Growth regulators
control, *Sitophilus oryzae* 1893
groundnuts, *Ephestia cautella* 976
sorghum, *Sitophilus oryzae* 1250
wheat flour, *Tribolium castaneum* 1582
Guam, poles, Isoptera 1773
Guavas
aflatoxins 1631
Aspergillus flavus, aflatoxins 1631
Aspergillus parasiticus, aflatoxins 1631
Attagenus unicolor, New Zealand, interceptions 1900
Botryodiplodia theobromae, enzymes 127
fungi, India 1628
Fusarium solani, India 1632
Phomopsis psidii, biochemistry 128
postharvest decay, coatings 1627
Sclerotium rolfsii 125

ms, production, whey 1379
alogenated hydrocarbons, wood preservatives 664
am, microbial count, heat treatment 557
ansenula anomala, control, preservatives 25
ay
Aspergillus, ammonium propionate 1477
fungi 1485
preservation, ammonium propionate 1476
CH
Ochlandra travancorica, insect pests 1729
timbers, insect pests 1766
CN, wood preservatives 239
ealth hazards, waste water treatment 1957
eat resistance, determination, spores, bacteria 726
eat treatment
ham, microbial count 557
mangoes
Botryodiplodia theobromae 1015
Glomerella cingulata 1015
milk, *Salmonella* 1718
peaches, *Ceratitis capitata* 1010
pears, storage decay 506
Phaseolus vulgaris, Acanthoscelides obtectus 146
wheat
pest control 466
Rhyzopertha dominica 1557
eating, cereals, *Rhyzopertha dominica* 1504
eating systems, bacteria, USSR 1225
elenalin, antifeedants, stored products pests 730
elicostylum pulchrum, beef, antibacterial properties 252
elminthosporium
mangoes
controlled atmosphere storage 1014
prochloraz 1014
emicelluloses
acetone-butanol, production 1333
biodegradation 789, 841
2,3-butanediol, production 1333
utilization
Clostridium acetobutylicum 1333
Klebsiella pneumoniae 1333
emp
insects
Burma 1728
New Zealand 1728
Thailand 1728
erbaria, *Idaea bonifata* 687
ermetic sealing, maize, insects 747
eterobostrychus aequalis
canes and rattans 1162
Ochlandra travancorica, control 1729
timbers, South Africa, interceptions 211
evea brasiliensis
Phellinus noxius, lignin, degradation 1758
Rigidoporus lignosus, lignin, degradation 1758
exahydro-1,3,5-triethyl-*s*-triazine
biocides 1945
mode of action 1945
resistance, *Pseudomonas putida,* genetics 1946
ides, *Euryomma peregrinum,* New Zealand 196
istamine, fish, estimation 1057
istorical buildings
Anobium punctatum
German Democratic Republic 1769
ionizing radiation 1769
fumigation, HCN 239
Hylotrupes bajulus, phosphine 688
ives, wood preservation 1792
omeopathic drugs, control, aflatoxins 1459
omes, fungi, Sweden 240
oney, bacteria, Fiji 88
ormoconis resinae, taxonomy 1844
orse manure, utilization, *Agaricus bisporus* 321
Humicola grisea* var. *thermoidea, enzymes, endoglucanases 1845
Hungary, feeds, mycotoxins 1487-1488
Hydrocarbons
biodegradation
Candida 1351
Pseudomonas aeruginosa 1348
Hydrocotyle umbellata, waste water treatment 378
Hydrocyanic acid
cotton
Anthonomus grandis 1158
Anthonomus vestitus 1158
Sacadodes pyralis 1158
Hydrogen, production, bacteria 1295
Hydrogen cyanide, control, insect pests 1290
Hydrogen peroxide
milk
bacteria 1701
microbial flora 580
psychrotrophic bacteria 188
Hylotrupes bajulus
control
cypermethrin 660
HCN 239
historical buildings, phosphine 688
Hyphoderma mutatum
control, PCP 1209
timbers 1209
Hypholoma fasciculare
Fagus 650
wood, growth 1173
Hypothenemus hampei, coffee, Jamaica 1589
Hyptis spicigera, insecticidal properties 738
Ice, fish, microbial count 150
Ice cream
containers, microbial count 684
Enterobacteriaceae
Egypt 1095
isolation 177
fungi, Egypt 1094
microbial flora
Greece 1084
Italy 1681
Staphylococcus aureus
enterotoxins 871
Argentina 1679-1680
yeasts, Egypt 1094
Idaea bonifata, dried plants 687
Imazalil
apples
Botrytis cinerea 509
Penicillium expansum 509
Capsicum annuum, storage decay 528
melons, storage decay 1031
pears, storage, diseases 1605
Immunoassay, *Clostridium botulinum,* toxins 407
Immunological techniques
foods
microbial contamination 1439
Salmonella 396
Improved wood
Kalotermes flavicollis, resistance 1770
Reticulitermes lucifugus, resistance 1770
Reticulitermes santonensis, resistance 1770
India
books, biodeterioration, fungi 1831
Cajanus cajan, Callosobruchus 964, 966-967
cereals
Laemophloeus 926
Rhyzopertha dominica 926
Sitophilus oryzae 926
Sitotroga cerealella 926
Tribolium castaneum 926
Cicer arietinum, Callosobruchus 964, 966-967
confectionery, microbial flora 1090
cotton, pest control 1158
cottonseed, *Ephestia cautella* 973
culture collection catalogues 1405
dried fruit, insect pests 853
feed grains, toxinogenic fungi 1480
flours
Callosobruchus 926
India *cont.*
flours *cont.*
Ephestia cautella 926
foods, mycotoxins 1465
fruits, fungi 1599
guavas
fungi 1628
Fusarium solani 1632
Lablab purpureus, Geotrichum candidum 1657
milk, *Bacillus* 1113
milk products, *Staphylococcus* 1091
paintings, fungi 1830
pest control 1280
pomegranates, postharvest decay 1626
postharvest systems, education 1423
potatoes
arthropod pests 1041
Cercospora solanicola 1652
pulses
Callosobruchus 926
Ephestia cautella 926
rice, storage 941
rice straw, biodegradation 1956
rodenticides, brodifacoum 274
sewage, land application 1991
sorghum, insect pests 1538
spices, insect pests 853
stored products, Coleoptera 1252
structural wood
Cryptotermes domesticus 654
Cryptotermes dudleyi 654
timbers
Cerambycidae 1249
Irpex lacteus 1742
Isoptera 1903
Trametes trogii 1742
vegetables
fungi 1599
postharvest decay 498
Vigna mungo, Callosobruchus 964, 966-967
Vigna radiata, Callosobruchus 964, 966-967
Volvariella diplasia, cultivation, rice straw 815
watermelons, fungi 1640
wood preservation 1779
Indoles
biodegradation, *Alcaligenes* 335
shrimps, bacteria 151
Indonesia
foods, aflatoxins 902
stored products
arthropod pests 1246
insects 1246
Industrial microbiology, books 1409
Industrial wastes
coke, reviews 2063
cyanides, reviews 2032
denitrification 1358
landfill 2008
sea pollution, metals 306
steel, reviews 2063
tipping, United Kingdom 327
Infant foods, mycotoxins 427
Infant formulae
coliform bacteria 595
microbial flora 1083
Egypt 180
preservation, lactoperoxidase system 1121
Staphylococcus, Nigeria 1673
Streptococcus, heat resistance 564
Infrared radiation
cereals, insect pests 1505
milk, microbial contamination 1705
Inonotus rheades, timbers, wood chemistry 1172
Inonotus weirii
culture medium 1270
wood chips 1270
Insect pests
books, Cameroon 835
cereals
Canada 58
cold resistance 752
control 453
detection, staining 928
infrared radiation 1505
integrated control 288

Insect pests *cont.*
cereals *cont.*
Israel 1506
microwave radiation 1505
monitoring 1506
Poland 454
resistance 1502
reviews 1502
spread 6
traps 714
United Kingdom 453
vacuums 1505
control, pheromones 282
cowpeas, reviews 1565
dried fruit, India 853
foods, New Zealand 852
forecasting, sex attractant traps 265
fruits
irradiation 496
New Zealand 852, 995
quarantine certification, USA 497
groundnuts, USA, Georgia 1587
logs, interceptions, Korea Republic 655
maize
insecticides 1521
Nigeria 1521
military rations 855
packaging 837
rice, Taiwan 67
sorghum
Africa 1537
Australia 1540
India 1538
South East Asia 1539
soyabeans
Israel 1506
monitoring 1506
prediction 1562
spices, India 853
stored products, monitoring 263
timbers
Australia, books 1402
Austria, books 1403
books 840
control 199
Korea Republic, interceptions 214-215
vegetables
irradiation 496
quarantine certification, USA 497
wheat
heat treatment 466
sampling techniques 1551
Turkey 471
ultrasound 732
Insecticides
control, stored products pests, reviews 1918
degradation, South East Asia 751
losses
cereals 751
legumes 751
nuts 751
neem extracts 284
sorghum
Cryptolestes ferrugineus 1567
Oryzaephilus surinamensis 1567
Rhyzopertha dominica 1567
Tribolium castaneum 1567
Trogoderma granarium 1567
Insects
cereals
carbon disulphide 748
integrated control 749
phosphine 748
control, protective atmospheres 720
culinary herbs 989
grain stores, United Kingdom 920
hemp
Burma 1728
New Zealand 1728
Thailand 1728
maize
fumigants 747
hermetic sealing 747
oats, estimation 1517
spices 989
stored products, Indonesia 1246
timbers 1161
control 1768
Insects *cont.*
wheat
carbon disulphide 746
carbon tetrachloride 746
Integrated control
cereals
insects 749
pest control 288
Interceptions, logs, insect pests, Korea Republic 655
Intravenous fluids, microbial contamination 1218
Iodine
fruits
postharvest decay 1598
prestorage treatment 510
Ionizing radiation
apple juice, *Byssochlamys fulva* 494
timbers, *Anobium punctatum* 1769
Iprodione
fungicide tolerance, *Alternaria mali* 300
pears, storage, diseases 1605
Ips typographus, timbers, lime (mineral) 1772
Iran, food poisoning, *Staphylococcus aureus* 1426
Iraq
cultured milks, microbial flora 1672
dairy products, microbial flora 181
ewe milk, microbial flora 1672
maize
mycotoxins 1522
toxinogenic fungi 1522
milk products, microbial flora 1676, 1683
sour milk, microbial flora 169
yoghurt, microbial flora 1672
Irpex flavus, timbers, resistance 647
Irpex lacteus
timbers 633
India 1742
Irradiation
bacon, *Clostridium botulinum* 1069
foods, New Zealand 1428
prawns, bacterial count 1060
Irrigation equipment, sugarcane fields, Formicidae, USA, Hawaii 1824
Ischnoderma resinosum
lignin 209
ligninolytic microorganisms 209
timbers, wood chemistry 200
wood, enzyme activity 653
Isolation technique, *Yersinia,* meat 543
Isoptera
control, plant extracts 1287
poles
Guam 1773
wood preservation 1773
timbers
camphor 1242
dichlorvos 1242
India, Gujarat 1903
insecticides 1765
malathion 1242
Saudi Arabia 1765
susceptibility 217
Tanzania 1738
wood preservation 658
Israel
cereals, insect pests, monitoring 1506
soyabeans, insect pests, monitoring 1506
Italy
books, biodeterioration 1826
cheeses
aflatoxins 571
Salmonella typhimurium 1425
ice cream, microbial flora 1681
marble, biodeterioration 1823
milk, aflatoxins 1143
Ivory Coast, yams, insect pests 1647
Jamaica, coffee, *Hypothenemus hampei* 1589
Japan
cereals
deoxynivalenol 459
nivalenol 459
coffee, toxinogenic fungi 486
foods
aflatoxins 902
Japan *cont.*
foods *cont.*
mycotoxins 418, 421, 888
ginger
Phytosciara zingiberis 987
Psilosciara flammulinae 987
interceptions
timbers
Platypodidae 656, 1248
Scolytidae 656, 1248
milk products, bacterial count 179
poultry meat, bacterial count 170
stored products, insect pests 1891
wheat, trichothecin 1561
Jatropha curcas, potatoes, *Phthorimaea operculella* 1651
Java, milk, *Campylobacter fetus* subsp. *intestinalis* 183
Jodfenphos
sorghum
Cryptolestes ferrugineus 1567
Oryzaephilus surinamensis 1567
Rhyzopertha dominica 1567
Tribolium castaneum 1567
Trogoderma granarium 1567
Jute, *Macrophomina phaseolina,* fibre quality 1732
Kalotermes brouni, timbers, New Zealar 213
Kalotermes flavicollis, improved wood, resistance 1770
Kefir, *Lactobacillus kefir* 182
Kenya
cassava, *Prostephanus truncatus* 189
maize, *Prostephanus truncatus* 1520, 1892
17-Ketosteroids, sugar factory waste, cor version 812
Khoa
microbial count 566
microbial flora, packaging 1088
Kidneys as food
ochratoxins 1062
ELISA 1064
Poland 1074
United Kingdom 1064
Kinetics, biodegradation 304
Klebsiella
milk products, Egypt 1095
phosphorus, release 804
waste water treatment 768
Klebsiella aerogenes
cadmium, detoxification 802
lead, detoxification 802
mercury, detoxification 802
Klebsiella pneumoniae
2,3-butanediol, production 790
cellulose, biodegradation 790
hemicelluloses, utilization 1333
oysters, health hazards 1663
whey, ultrafiltration permeate, utiliza tion 776
Klebsiella pneumoniae* subsp. *ozaenae,
bromoxynil, biodegradation 2025
Klockera apiculata, control, preservative 25
Kluyveromyces, whey, utilization 2046
Kluyveromyces bulgaricus, whey, lactose, hydrolysis 809
Kluyveromyces fragilis
whey
fermentation 1362, 1365, 1373, 2047, 2049-2050
protein concentrates, upgrading 7
utilization 1361, 1364, 1367, 1996 2042
Kluyveromyces lactis
whey
fermentation 1378
utilization 1364
Kluyveromyces marxianus, whey, fermen tation 2048
Kluyveromyces marxianus* var. *lactis, tax onomy 1844
Korea Republic
cereals, mycotoxins 890, 1513
interceptions
Bostrichidae, timbers 214
insect pests, timbers 215
logs, insect pests, interceptions 655

Korea Republic *cont.*
milk
bacterial count 1139
keeping quality 585
Kulfi, Enterobacteriaceae, isolation 177
Kuwait
municipal refuse disposal, composting 348
rodents, control 832
Lablab purpureus
Alternaria tenuissima 478
Callosobruchus maculatus, development 1570
Fusarium solani 478
Geotrichum candidum, India 1657
Laboratory tests, wood preservation, fungi 1912
Lachnocladiaceae, Europe, books 2082
Lactic acid
production
agricultural wastes 1320
whey 1369, 2045
Salmonella typhimurium, inhibition 176
veal, decontamination 551-552
Lactic acid bacteria, meat, mass spectrometry 167
Lactobacilli, foods, culture media 23
Lactobacillus
beef 162
cod 149
meat 165
identification 164
meat products, nisin 26
resistance, nisin 26
Lactobacillus buchneri
cheeses
health hazards 1152
histamine 1152
Lactobacillus bulgaricus
milk, hydrogen peroxide 1701
whey, utilization 1369
Lactobacillus casei
agricultural wastes, utilization 1320
lactose, biodegradation 1305
whey, fermentation, patents 810
Lactobacillus kefir, kefir 182
Lactoperoxidase system
buffalo milk, preservation 1695-1696
infant formulae, preservation 1121
milk
Campylobacter jejuni 1101
storage 611
Lactose
biodegradation
Acetobacterium woodii 1305
Lactobacillus casei 1305
Methanosarcina barkeri 1305
butanediol, production 1366
methane production 1305
Laemophloeus
cereals
India 926
Poland 454
Lagoons
monitoring 1982
waste water treatment, reviews 1980
Lakes, cellulolytic microorganisms, USSR 787
Lamb (meat), microbial count, chilling 548
Laminated wood, wood preservation 1797
Land application
municipal refuse disposal
plants 1312
soil properties 769
olive marc, agronomy 1989
potato waste 1308
sewage, India 1991
sewage sludge 318, 1994
heavy metals, plants 1310
mine spoil, revegetation 1309
plants 1307
USA 1993
tannery waste, plants 1383
waste waters
reviews 1992
soil properties 1990
wastes 1311, 1988
Landfill
biogas, production, reviews 1995
gas production 771
Landfill *cont.*
industrial wastes 2008
methane production 771
United Kingdom 770
wastes
medical 772
municipal refuse 772
Laser radiation, control, stored products pests 1887
Lasioderma serricorne
cassava, losses 1390
control
cypermethrin 291
deltamethrin 291
dill 1288
fenvalerate 291
insecticides 1934
phosphine 1923
plant extracts 1863
sorptive coatings 1930
spices 1244
tetrachlorvinphos 291
Coriandrum sativum 94
lentils, development 1563
military rations 855
stored products
Australia, New South Wales 1868
Japan 1891
tobacco
cooling 1591
methoprene 1590
Latheticus oryzae
control
fumigants 748
growth regulators 1275
lentils, development 1563
Lathridiidae, cereals, Canada 1491
Latin America, poles, preservation 1785
Lead
detoxification, *Klebsiella aerogenes* 802
effluents, absorption, *Galium* 360
oxidation, microorganisms 1226
Leather
Bacillus 628
bacteria, proteolysis 629
Leben, microbial flora, Morocco 1085
Lecythophora mutabilis, timbers, Cu-Cr-As 1759
Leeks
Botrytis 144
Botrytis porri, Canada 1054
storage
cold storage 1047
controlled atmosphere storage 1047, 1050
Legislation
foods, mycotoxins, USA 2
milk, bacterial count, German Federal Republic 598
mycotoxins, South Africa 35
Legumes, insecticides, losses 751
Lemons
Botryodiplodia theobromae, enzymes 127
Ceratitis capitata, resistance 117
Geotrichum candidum
potassium sorbate 522
sodium 2-phenylphenolate 522
Penicillium digitatum
fungicides 1621
packaging 1621
waxes 1621
storage decay
control 519, 1025
2-phenylphenol 523
Lentils
Callosobruchus chinensis, resistance 1568
stored products pests, development 1563
Lentinula edodes
ligninolytic microorganisms 1841
lignocellulose, biodegradation 1343
taxonomy 1844
timbers 1174
Lentinus cyathiformis, plywood, laboratory tests 639
Lentinus edodes, taxonomy 1844
Lentinus lepideus
poles, physiology 1752
timbers, creosotes 1750
Lepidoglyphus destructor, barley, control 933
Lepidoptera
cereals, biological control 923
control
abamectin 1885
biological control 1927
fenoxycarb 1919
microbial pesticides 923
sterile insect release 1926
Leptadenia reticulata, potatoes, *Phthorimaea operculella* 1651
Lesotho, wood preservation 1187
Lettuces
storage 1655
storage decay 1055
Leuconostoc, ewe milk, Bulgaria 1697
Leuconostoc oenos, fruit juices 101
Lichens, organic acids, stones, weathering 256
Lignin
biodegradation 791, 841, 1342, 1398
basidiomycetes 1331
estimation 351
fungi 641
Merulius tremellosus 341
Phanerochaete chrysosporium 197, 347, 376, 1385
enzymes 705
reviews 786
Ischnoderma resinosum 209
Phanerochaete chrysosporium
chemistry 1159
enzymes 1734
Poria medulla-panis 209
Xylobolus frustulatus 209
Ligninase
Phanerochaete chrysosporium 371
organochlorine compounds 333
waste water treatment, phenols 371
Ligninolytic microorganisms
basidiomycetes 351, 1331
Coriolus versicolor 1841
Ischnoderma resinosum 209
Lentinula edodes 1841
Merulius tremellosus 341
Phanerochaete chrysosporium 1841
Phlebia brevispora 1841
Pholiota mutabilis 1841
Pleurotus ostreatus 1841
Poria medulla-panis 209
Xylobolus frustulatus 209
Lignocellulose
biodegradation 791
basidiomycetes 1731
Lentinula edodes 1343
Streptomyces viridosporus 1733
ethanol, production, reviews 2015
fermentation 1334
Lime (mineral), timbers, wood borers 1772
Limnoria, marine structures, preservation 220
Limulus test
cheeses, Gram negative bacteria 565
milk
Gram negative bacteria 565
quality 574
reviews 573, 575
Lindane
control, insect pests 737
logs 225
maize, *Prostephanus truncatus* 936
resistance
Callosobruchus maculatus 710
Ephestia cautella 1864
insects 719
Oryzaephilus surinamensis 279
timbers, wood borers 1179
wood preservatives 659
Linifolin A, antifeedants, stored products pests 730
Linseed oil, wheat, *Sitotroga cerealella* 1283
Lipids
production
banana waste 361
Candida curvata 361, 363
Lipolytic bacteria, butter, culture media 1149
Lippia geminata, rice, stored products pests 1531

Liriomyza trifolii, celery, cold storage 1053
Listeria, milk, Spain 1131
Listeria monocytogenes
cabbages
pH 1656
sodium chloride 1656
temperature 1656
Litchi
Geotrichum candidum
captafol 1620
nickel chloride 1620
nickel sulphate 1620
Logs
control 225
insect pests
interceptions, Korea Republic 655
Korea Republic, interceptions 215
pest control, fumigation, ship holds 655
Platypus, New Zealand 1775
wood borers
Canada 1180-1181
control 1179
Low fat milk
psychrotrophic bacteria, sensory evaluation 1677
Salmonella typhimurium, food poisoning 1710
Lubricants, biodeterioration 1213
Lyctus brunneus, timbers, wood preservation 659
Lyctus linearis, timbers, wood preservation 659
Lyctus pubescens, timbers, wood preservation 659
Lyophyllum decastes, straw, fermentation 1318
Macrophomina phaseolina, jute, fibre quality 1732
Madeira, *Ommatoiulus moreletii,* distribution 257
Maize
aflatoxins 36, 41, 464, 1458, 1529
analysis 410
dust 1527
estimation 409
metabolism, fungi 901
Mexico 1526
Nigeria 938
Pakistan 64
zinc 1530
Aspergillus candidus, morphology 1528
Aspergillus flavus, temperature 1524
Aspergillus glaucus, storage decay 462
Aspergillus niger 901
Aspergillus sejunctus 901
citrinin, heat treatment 65
deoxynivalenol 457, 1525
diacetoxyscirpenol, inactivation 903
Ephestia cautella, population dynamics 1896
fungi, Saudi Arabia 456
fusarins 1484
Fusarium 1474
Fusarium solani 901
Gibberella zeae, survival 1525
insect pests
damage 57
insecticides 1521
Nigeria 1521
insects
fumigants 747
hermetic sealing 747
moniliformin 1484
mycotoxins 463, 1474
Fusarium 413
Iraq 1522
ochratoxins
Bulgaria 887
estimation 1469
moisture content 1523
Nigeria 938
Penicillium, storage decay 462
pest control, permethrin 63
Plodia interpunctella, microbial pesticides 1493
Prostephanus truncatus
control 936, 1520, 1892
Kenya 1520, 1892
storage losses, Tanzania 63
Tanzania 1892
Maize *cont.*
Prostephanus truncatus cont.
Togo 62
Rhizopus 901
secalonic acids 1529
Sitophilus granarius 716
Sitophilus oryzae
biology, development 1496
plant extracts 1937
vegetable oils 1519
Sitophilus zeamais
biology, development 1496
control 60
cyfluthrin 935
deltamethrin 289
fenitrothion 935
flucythrinate 61
gamma radiation 59
malathion 935
susceptibility 59
Sitotroga cerealella
flucythrinate 61
varietal susceptibility 937
T-2 toxin
detection 428
inactivation 903
toxinogenic fungi, Iraq 1522
α-zearalenol, determination 878
zearalenone 425, 1525
determination 878
Nigeria 938
sampling 441
Maize meal, fungi, culture media 17
Maize mills
pest control
application methods 1272
dichlorvos 1272
Maize silage, mycotoxins, Hungary 1487-1488
Malathion
apples, *Archips micaceanus* 503
cereals, stored products pests 1511
control
Ephestia cautella 1284
insect pests 737, 1282
Tribolium castaneum 713, 1935
maize
Prostephanus truncatus 936, 1520
Sitophilus zeamais 60, 935
Ochlandra travancorica, insect pests 1729
raisins, insects 1036
resistance
Callosobruchus maculatus 710
Cryptolestes ferrugineus 1851
Ephestia cautella 1864, 1897
insects 719
Oryzaephilus surinamensis 279-280, 1277
Rhyzopertha dominica 1245, 1901
Sitophilus zeamais 1901
stored products pests
Australia 1882
Canada 1921
Tribolium 753
Tribolium castaneum 717, 1245
rice, *Sitotroga cerealella* 1286
rye, *Cryptolestes ferrugineus* 1494
sesame
Oryzaephilus surinamensis 974
Tribolium castaneum 974
sorghum, *Sitophilus oryzae* 946
timbers, Isoptera 1242
triticale, *Cryptolestes ferrugineus* 1494
wheat
Cryptolestes ferrugineus 1494
insect pests 1547
Oryzaephilus surinamensis 921
residues 1555
Sitophilus granarius 921
Sitotroga cerealella 1283
Tribolium castaneum 921
Malaysia
beef, microbial count 547
cereals, storage 1495
chicken meat, microbial count 547
Malbranchea pulchella
cellulolytic microorganisms 1237, 1239
enzymes, cellulases 1237, 1239
Maleic hydrazide, postharvest treatment, onions 1648
Malt
Aspergillus fumigatus, Nigeria 1518
Aspergillus versicolor, Nigeria 1518
toxinogenic fungi 461
Mancozeb
control, rice, discoloration 1535
mandarins, postharvest treatments 165
Mandarins
Alternaria citri 517
Parlatoria pergrandii, control 1017
postharvest treatments
boric acid 1625
mancozeb 1625
Mangoes
Alternaria alternata 119
Anastrepha ludens, quarantine 1624
Aspergillus niger, control 516, 520
Botryodiplodia theobromae
enzymes 127
heat treatment 1015
postharvest treatment 1622
Dacus dorsalis, ethylene dibromide 1020
Dacus tryoni, dimethoate 118
Dothiorella dominicana, Nigeria 162
fungi
controlled atmosphere storage 101
gamma radiation 1618
physiology 106
prochloraz 1014
Glomerella cingulata
heat treatment 1015
postharvest treatment 1622
Leuconostoc oenos 101
packing, containers 116
prestorage treatment, benomyl 116
Rhizopus arrhizus, control 520
transport, containers 116
Marble, biodeterioration, Italy 1823
Marine borers, marine structures, preservation 220
Marine fouling, reviews 694
Marine fungi, timbers, USSR 1177
Marine structures
biofouling
control 1836
reviews 694
marine borers, preservation 220
preservation 1801
fumigants 1796
wood preservation 222
Mass spectrometry, lactic acid bacteria, meat 167
Materials testing, fungi, growth 5
Meat
aflatoxins, detection, ELISA 437
bacterial count 1067
ATP 556
Campylobacter coli, USA 160
Campylobacter jejuni
culture media 18
USA 160
Flavobacterium 165
fungi, culture media 17
lactic acid bacteria, mass spectrometry 167
Lactobacillus 165
identification 164
microbial contamination, estimation, ATP 1666
microbial count, determination 865
microbial flora 1665
Micrococcus 165
ochratoxins
detection 1061
ELISA 1064
extraction 900
Poland 1074
United Kingdom 1064
Pseudomonas 165
taxonomy 1233
Pseudomonas lundensis 1234
quality, storage 1063
Salmonella
Brazil 1073
ELISA 1664
Staphylococcus, enterotoxins 1446
Staphylococcus aureus, isolation 1071
Yersinia, isolation technique 543

leat products
Clostridium botulinum, phosphates, reviews 1668
Clostridium perfringens
cooking 1065
microwave cooking 1065
Lactobacillus, nisin 26
microbial contamination, estimation, ATP 1666
microbial count
Canada 1667
cooking 1065
microwave cooking 1065
nisin 1070
Saudi Arabia 550
Streptococcus lactis 1070
microbial flora 155
culture media 399
leatworks effluent
anaerobic digesters, Chile 362
treatment, reviews 2036
water pollution, rivers 302
legatoma graeseri, cereals, China 1509
lelanocarpus albomyces, xylanase, production, bagasse 818
lelons
Dacus xanthodes, New Zealand, interceptions 1899
postharvest decay, USA 1639
storage dips 1031
lercuric chloride, fruits, postharvest decay 1598
lercury, detoxification, *Klebsiella aerogenes* 802
leruliopsis corium, timbers 633
lerulius tremellosus
lignin, biodegradation 341
ligninolytic microorganisms 341
lesua nagassarium
decay fungi, resistance 647
resistance 647
letallic naphthenates, wood preservatives 1812
letals
bacteria, seawater 1220, 1222
bacterial leaching 353
reviews 795
Bacterium halophilum, seawater 1222
Bacterium singulare, seawater 1222
biofilms, *Pseudomonas atlantica* 693
extraction
Aspergillus niger 1346
Trichoderma harzianum 1346
Pseudobacterium boreale, seawater 1222
sea pollution, industrial wastes 306
letalworking fluids
Acinetobacter 1819
bacteria, pasteurization 1818
Pseudomonas 1819
lethacrifos
barley, *Lepidoglyphus destructor* 933
control
Ephestia cautella 1264, 1284
insect pests 1282
maize, insect pests 1521
sesame
Oryzaephilus surinamensis 974
Tribolium castaneum 974
wheat
Oryzaephilus surinamensis 921
pest control 75
Sitophilus granarius 921
Tribolium castaneum 921
lethane production
bark 315
biomass 325
cellulosic wastes, anaerobic digestion 2014
China 1962
lactose 1305
landfill 771
United Kingdom 770
municipal refuse disposal, anaerobic digestion 2007
refuse, reviews 310
technology, USA 323
waste waters, reviews 309
whey 765, 2051-2052
lethanobacteriaceae
anaerobic digesters 767
Methanobacteriaceae *cont.*
anaerobic treatment 317
sludges 314
identification, antibodies 369
Methanosarcina, anaerobic digesters, identification 766
Methanosarcina barkeri, lactose, biodegradation 1305
Methomyl, potatoes, *Phthorimaea operculella* 140
Methoprene
oats, stored products pests 1516
tobacco, *Lasioderma serricorne* 1590
3-Methoxy-4-hydroxycinnanate, biodegradation, *Enterobacter* 792
Methoxychlor
timbers, wood borers 1179
wood preservatives 659
Methyl bromide
asparagus, postharvest decay 1051
cereals
Rhyzopertha dominica 1492
Sitophilus oryzae 1492
Tribolium castaneum 1492
control
Callosobruchus chinensis 281
insect pests 1290
insects 740, 745
Rhyzopertha dominica 1254
stored products pests 295
Tribolium castaneum 728
Trogoderma granarium 271
cucumbers
Dacus cucurbitae 1638
Dacus dorsalis 1638
Quercus, Ceratocystis fagacearum 1774
timbers, fungi 224
wheat
insect pests 286
Sitophilus granarius 1549
wood preservatives 1774
Methyl chloroform
control
insect pests 736
Trogoderma granarium 271
Methyl formate, control, *Callosobruchus chinensis* 281
Methyl iodide, control, *Callosobruchus chinensis* 281
Methyl isocyanate, preservation, poles 1808
Methyl isothiocyanate
poles, fungi 1203
timbers, *Poria carbonica* 677
wood preservatives 1203
α-Methyl styrene, biodegradation, *Pseudomonas acidovorans* 1345
Methylchloroform, control, insect pests 1290
Methylene bisthiocyanate, wood preservatives 1804
Metomeclan
apples
Botrytis cinerea 509
Penicillium expansum 509
Mexico
maize, aflatoxins 1526
milk products, *Brucella* 1675
nuts, aflatoxins 484
Microbial contamination
confectionery 89
dairy products 89
foods
estimation 860
immunological techniques 1439
intravenous fluids 1218
milk 1687, 1700
UHT milk, detection 185
Microbial count
beef 544
chilling 548
culture media 545
Malaysia 547
buffalo milk 566
chicken meat, Malaysia 547
dried foods, techniques, reconstitution 402
foods, safety 392
ham, heat treatment 557
ice cream, containers 684
Microbial count *cont.*
khoa 566
lamb (meat), chilling 548
meat, determination 865
meat products
cooking 1065
microwave cooking 1065
nisin 1070
Saudi Arabia 550
Streptococcus lactis 1070
milk 566
resazurin test 601
pork, chilling 548
spices
determination 865
Pakistan 988
vegetables, Egypt 538
Microbial flora
buffalo milk, sterilization 1136
cakes, France 970
cheeses 1153
Bulgaria 1155
cold storage 624
chhana, packaging 1089
confectionery, India 1090
Cottage cheese, sorbic acid 1157
crab meat 148
cream 563
cultured milks, Iraq 1672
dahi 570
ewe milk, Iraq 1672
foods
conferences 1393-1394
culture media 399
Morocco 849
techniques 16
goat milk 602
milk quality 578
United Kingdom 186
ice cream, Italy 1681
infant formulae, Egypt 180
khoa, packaging 1088
meat products 155
milk 563, 1117, 1122, 1134
cold storage 587
cooling 1704
Greece 605
heat treatment 1097
hydrogen peroxide 580
quality 1138
milk products 175, 1086
food poisoning 1685
Iraq 1676, 1683
reconstituted milk, ultrafiltration 583
sausages 155
yoghurt, Iraq 1672
Microbial pesticides
cereals, *Plodia interpunctella* 1493
raisins
Ephestia 1036
Plodia interpunctella 1036
wheat, insect pests 1547
Microbial protein, economics 1998
Microcerotermes, timbers, Saudi Arabia 1765
Microcerotermes bresoni, timbers, acetylation 1780
Micrococcus
cheeses, identification 1726
ewe milk, Bulgaria 1697
marble, biodeterioration 1823
meat 165
Micrococcus luteus, pyridines, biodegradation 2011
Microflora, black pepper, moisture content 1592
Microorganisms
bacterial leaching, identification, genetics 2005
enumeration, tomato concentrate, analytical methods 130
feeds, production 773
foods, production 773
lead, oxidation 1226
polymers, resistance 681
soil, air pollution, decomposition 305
toxins, foods, conferences 2075
Microtermes
timbers
Saudi Arabia 1765
susceptibility 217

Microwave radiation, cereals, insect pests 1505
Military rations, insect pests 855
Milk
aflatoxins 190, 1096, 1146, 1148, 1686
analysis 411
detection 615
determination 877
estimation 614, 1145
ELISA 1144
liquid chromatography 616
immunoassay 1723
Italy 1143
metabolism 412
processing 1674
storage 612
ultraviolet radiation 191
United Kingdom 1147
Bacillus
germination 581
India 1113
lipolysis 1112
UHT treatment 586
Bacillus cereus, keeping quality 1714
Bacillus subtilis, heat treatment 1114
bacteria
hydrogen peroxide 1701
identification 1724
plate count 561
bacterial count 610, 1115, 1124
ATP
determination 1137
impedance 178
Bactoscan 1142
cheeses, sensory evaluation 1715
culture media 1713
DEFT 590, 1688
German Federal Republic 1711
Japan 179
keeping quality 582
Korea Republic 1139
legislation, German Federal Republic 598
methodology 1703
methylene blue test 1140
Poland 1126
resazurin test 1699
sterilization 1712
techniques 1720
USA 1128
Brucella, Mexico 1675
Campylobacter fetus subsp. *intestinalis,* Java 183
Campylobacter jejuni
France 851
health hazards 1116
lactoperoxidase system 1101
Clostridium 1092
Clostridium tyrobutyricum
culture media 577, 1105
determination 1717
coliform bacteria 1120
Poland 1129
deoxynivalenol, estimation 1722
endotoxins, Limulus test 1678
enterotoxins, *Escherichia coli* 559
Escherichia coli 592
culture media 1133
formaldehyde 1692
inhibition 1110
reactivation 1099
volatile compounds 1689
Flavobacterium 1119
fungi 589
Gram negative bacteria 606, 609
culture media 1721
Limulus test 565
keeping quality 563
copper 1719
estimation
bacterial count 1707
endotoxins 1707
impedance 1707
heat treatment 1702
Korea Republic 585
lactoperoxidase system 1135
pasteurization 1719
plate count 576
Listeria, Spain 1131
microbial contamination 1687, 1700
health hazards 1111, 1125
Milk *cont.*
microbial contamination *cont.*
infrared radiation 1705
ultraviolet radiation 1705
microbial count 566
milk payments 1108
resazurin test 601
microbial flora 187, 563, 568-569, 1117, 1122, 1134
cold storage 587
cooling 1704
Greece 605
heat treatment 1097
hydrogen peroxide 580
quality 1138
standards 174
mycotoxins, Poland 613
post pasteurization contamination 189, 584, 600, 1115, 1122
health hazards, USA 1716
psychrotrophic bacteria 579
reviews 1104
preservation, potassium nitrite 1693
Pseudomonas 1118
Pseudomonas fluorescens 607
psychrotrophic bacteria
amino acids 1690
cold storage 588, 1694
detection 1102
Egypt 1127
gelation 1103
hydrogen peroxide 188
nisin 1690
oxygen, tension 1708
potassium sorbate 188
proteinases 1141, 1232
sensory evaluation 1677
quality 599
Limulus test 574
reviews 573, 575
UHT treatment 1100, 1130
Salmonella
German Federal Republic 1684
heat treatment 1718
reviews 1684
Salmonella typhimurium
food poisoning 596-597, 1710
formaldehyde 1692
Shigella sonnei, culture media 1133
Staphylococcus 1123
culture media 1107
heat treatment 1097
Staphylococcus aureus, formaldehyde 1692
storage
lactoperoxidase system 611
methylene blue test 608
Streptococcus 1123
Streptococcus lactis, formaldehyde 1692
Streptococcus thermophilus, formaldehyde 1692
T-2 toxin, estimation 34
testing, techniques 1109
thermoduric bacteria 593
Egypt 1127
Yersinia
classification 1231
culture media 1709
Yersinia enterocolitica
Bulgaria 1132
enterotoxins 870
France 1706
virulence 697
Milk products
aflatoxins 173, 1686
bacteria, plate count 561
bacterial count, Japan 179
coliform bacteria 560
culture media 1671
endotoxins, Limulus test 1678
Enterobacter aerogenes 567
Enterobacteriaceae 560
Egypt 1095
isolation 177
enterotoxins, *Escherichia coli* 559
Escherichia coli 560, 567
fungi, Egypt 1094
Geotrichum candidum, morphology 1235
Lactobacillus kefir 182
Milk products *cont.*
microbial flora 175, 1086
culture media 399
food poisoning 1685
India 1090
Iraq 1676, 1683
packaging 1089
psychrotrophic bacteria
enzymes 1087
sensory evaluation 1677
quality 1081
Salmonella
German Federal Republic 1684
New Zealand 562
reviews 1684
Staphylococcus, India 1091
Staphylococcus aureus
detection 397
enterotoxins 175
yeasts, Egypt 1094
Milk quality, Cottage cheese, manufactu 174
Milk substitutes, fungi 907
Milk testing
analytical methods 1098
conferences 2074
Millets
arthropod pests 1542
Corcyra cephalonica, moisture conten 455
insect pests, damage 57
Mills
Ephestia cautella, dichlorvos 1272
Tribolium castaneum, dichlorvos 127
Mine spoil
biodegradation, bacteria 2061
revegetation, sewage sludge, land app cation 1309
uranium, bacteria, biodeterioration 2031
Minthea rugicollis, Ochlandra travancor ica, control 1729
Mites
cereals, Canada 1507
feeds
detection 1472
determination 400
enumeration 1269
physical control 1473
United Kingdom 909
foods, Egypt 1268
stored products
biological control 1905
China 1907
Egypt 1908
taxonomy 1906
MO-70616, control, *Lasioderma serricorn* 1934
Models, activated sludge 1975
Molasses
feeds, production 2033
fermentation, propionic acid bacteria 2033
lactic acid, production 1320
residues, utilization, *Pseudomonas* 138
utilization
Lactobacillus casei 1320
reviews 364
vitamin B12, production 2033
Monilinia fructicola, peaches, biological control 1617
Monilinia laxa
stone fruits 1616
South Africa 1615
Monitoring, stored products, insect pests 263
Monochamus galloprovincialis, timbers, control 1179
Monodictys pelagica, beech 652
Moraxella, cod 149
Morchella crassipes, whey, utilization 2046
Morocco
apples, *Alternaria alternata* 504
foods, microbial flora 849
leben, microbial flora 1085
Mothproofing, wool textiles 679
Mozambique, wood preservation 1187
Mucor
beet pulp, utilization 1315
control, essential oils 1240, 1952

***Mucor** cont.*
feeds, USSR 911
milk products, Egypt 1094
proteins, production 1315
Mucor mucedo, beef, antibacterial properties 252
Mucor piriformis
control
heat treatment 506
sodium 2-phenylphenolate 506
packhouses, pome fruits 507
pears 1610
resistance, benomyl 507
Mung beans
Callosobruchus analis, neem extracts 959
Callosobruchus chinensis 78
gamma radiation 474
neem extracts 922, 959
plant oils 961
Pongamia glabra 922
varietal susceptibility 80
Callosobruchus maculatus 78
neem extracts 959
plant oils 961
Municipal refuse disposal
anaerobic digestion
methane production 2007
rumen microorganisms 2007
composting, Kuwait 348
land application
plants 1312
soil properties 769
landfill 772
Municipal wastes
composting, USA 1967
sewage sludge, composting, temperature 763
Murals
Beauveria alba
p-chloro-*m*-cresol 1829
phenylmercuric acetate 1829
United Kingdom 1829
Museum specimens
Bacillus 628
preservation, antiseptics 1828
Mussels
bacteria 147
Clostridium perfringens, United Kingdom 542
Escherichia coli, United Kingdom 542
Streptococcus faecalis, United Kingdom 542
Mustard
groundnuts, *Aspergillus flavus* 980
maize, *Sitophilus oryzae* 1937
Mustard oil, wheat, *Sitotroga cerealella* 1283
Myceliophthora thermophila
cellulolytic microorganisms 1387
enzymes, cellulases 1387
Mycosphaerella, cucumbers, handling, techniques 1030
Mycotoxins
aflatoxins
Amaranthus 880
apple juice, potassium sorbate 895
Aspergillus flavus, genetics 415
buckwheat, analysis 410
cassava 424
cereals, Ethiopia 929
cheeses 626-627, 1096
analysis 410
Italy 571
chicken meat 1079
coffee 487-488
estimation 982
control
carbon monoxide 40
homeopathic drugs 1459
cottonseed 41
detection 1450
cottonseed oilmeal, decontamination 482
cowpea meal
temperature 477
water content 477
cowpeas
temperature 477
water content 477
cream 1096
Mycotoxins *cont.*
aflatoxins *cont.*
detection 891
dried milk, estimation 614
feeds 447, 449, 892, 912
detection 916, 1481
ELISA 437
liquid chromatography 426
estimation 1482
moisture content 42
Spain 1467
temperature 42
Turkey 915
Venezuela 913-914
foods 46, 892
Burundi 879
chemistry 886
detection, liquid chromatography 426
ELISA 38
Indonesia 902
Japan 902
Philippines 902
Spain 1467
USSR 438
ginger, detection 1450
groundnuts 36
analysis 410
detection 979, 1450
gamma radiation 1588
hygroscopicity 92
Singapore 37
storage 978
guavas 1631
maize 36, 41, 464, 1458, 1529
analysis 410
dust 1527
estimation 409
metabolism, fungi 901
Mexico 1526
Nigeria 938
Pakistan 64
zinc 1530
meat, detection, ELISA 437
milk 190, 1096, 1146, 1148, 1686
analysis 411
detection 615
determination 877
estimation 614, 1145
ELISA 1144
liquid chromatography 616
immunoassay 1723
Italy 1143
metabolism 412
processing 1674
storage 612
ultraviolet radiation 191
United Kingdom 1147
milk products 173, 1686
nut products, United Kingdom 972
nuts, Mexico 484
olive oil 485
olives 485
orange juice, bioassays 889
pulses 1575
raisins, drying 1646
shrimps, pastes, Singapore 37
soyabeans 36, 41, 1581
spices 95
Alternaria, reviews 43-44
alternariol methyl ether, tomatoes, estimation 1642
analysis
books 844
immunological techniques 423
animal products 1463
Aspergillus, butter 617
Aspergillus clavatus, reviews 1456
Australia 446
beans, Yugoslavia 479
books 843, 845
Canada, books 904
cereals
China 1461
Korea Republic 890, 1513
Nigeria 1546
South Africa 875
Taiwan 1461
United Kingdom 1514
USSR 1461
cheeses 192
Mycotoxins *cont.*
citrinin
barley
estimation, liquid chromatography 431
temperature 934
feeds
detection 897
estimation 450
foods 46
maize, heat treatment 65
wheat, estimation, liquid chromatography 431
conferences 829, 831
control
diacetyl 1452
potassium sorbate 33
cyclopiazonic acid 445
deoxynivalenol
analysis 422
biosynthesis 47
bread 414, 480
cereals 458
detection 930, 1471, 1515
estimation 45, 881
gas chromatography 432
Japan 459
United Kingdom 434
estimation, immunoassay 1466
feeds
Canada 1464
USA 1464
foods
Canada 1464
USA 1464
maize 457, 1525
milk, estimation 1722
wheat 414, 457, 480
distribution, milling 956
sodium bisulphite 1560
USA 955
detection
bioassays 442
liquid chromatography 885
diacetoxyscirpenol
feeds, bioassays 1486
maize, inactivation 903
estimation 894
reviews 882, 1455
feeds 56, 420, 452, 1451, 1474
China 1461
Finland 39
German Federal Republic 910
Hungary 1487-1488
reviews 50
South Africa 875
Taiwan 1461
United Kingdom 451
USSR 1461
foods 420, 1451
China 1461
fungi 416
German Federal Republic 884
India 1465
Japan 418, 421, 888
legislation, USA 2
reviews 50
Taiwan 1461
USSR 1461
fusarins, maize 1484
Fusarium, foods 413
Fusarium tricinctum 896
Gibberella zeae 896
health hazards 1462
infant foods 427
kojic acid, detection, bioassays 1457
legislation, South Africa 35
maize 463, 1474
Iraq 1522
milk, Poland 613
moniliformin
maize 1484
production, *Fusarium* 1454
nivalenol
cereals
detection 930
estimation 881
Japan 459
nuts 483

Mycotoxins *cont.*
ochratoxins
barley
estimation 435
liquid chromatography 431
radioimmunoassay 460
temperature 934
beans, Bulgaria 887
detection, bioassays 906
eggs 1080
feeds 919
detection 897
estimation 435, 450
foods, estimation 1469
kidneys as food 1062
Poland 1074
maize
Bulgaria 887
moisture content 1523
Nigeria 938
meat
detection 1061
ELISA 1064
extraction 900
United Kingdom 1064
olive oil 485
olives 485
poultry meat 1080
wheat
Denmark 957
estimation, liquid chromatography 431
patulin
apple juice 493
determination 876
United Kingdom 993
cereals
moisture content 931
temperature 931
detection, bioassays 1457
foods 46, 49
grape juice 100
pecans 91
research 439
Netherlands 51
reviews 1453
Rhizopus 54
rubratoxins, detection, bioassays 906
secalonic acids, maize 1529
sterigmatocystin
barley, immunoassay 444
cheeses 1686
estimation 1725
feeds, estimation 905
T-2 toxin
detection, bioassays 906
feeds, bioassays 1486
maize
detection 428
inactivation 903
milk, estimation 34
tenuazonic acid, tomatoes, estimation 1642
thin layer chromatography, reviews 55
trichothecenes
detection 53, 433
bioassay 898
estimation 52, 430
gas chromatography 429
feeds, estimation 1460
foods, estimation 1460
wheat 899
trichothecin
grape juice 100
detection 992
wheat, Japan 1561
wines, detection 992
viomellein, barley 436
wheat 440
Poland 472
xanthomegnin, barley 436
α-zearalenol
detection 893
maize, determination 878
zearalenone
detection 893
ELISA 417
feeds 425
maize 425, 1525
determination 878
Nigeria 938

Mycotoxins *cont.*
zearalenone *cont.*
maize *cont.*
sampling 441
reviews 1470
soyabean oilmeal, sampling 441
Myocalandra exarata, Ochlandra travancorica, control 1729
Myrothecium roridum, watermelons, India 1640
Namibia, wood preservation 1187
Naphthalene
biodegradation
Pseudomonas 797
Pseudomonas fluorescens 1350
Pseudomonas putida 1350
Necrobia rufipes
dried fish
deltamethrin 1056
pirimiphos-methyl 1056
tetrachlorvinphos 1056
Nectarines
Botrytis cinerea 1011
Penicillium expansum 1011
Rhizopus stolonifer 1011
Neem extracts
control, *Corcyra cephalonica* 1274
mung beans
Callosobruchus analis 959
Callosobruchus chinensis 922, 959
Callosobruchus maculatus 959
pearl millet, *Ephestia cautella* 943
sorghum, *Sitophilus oryzae* 922
wheat
Rhyzopertha dominica 284
Sitotroga cerealella 1283
Nesogordonia papaverifera, control, repellents 1242
Netherlands
foods
Staphylococcus, enterotoxins 1446
Yersinia 1431
mycotoxins, research 51
New Zealand
culture collection catalogues 1392
foods
insect pests 852
irradiation 1428
fruits, insect pests 852, 995
hemp, insects 1728
hides, *Euryomma peregrinum* 196
interceptions
cashews, *Suidasia pontifica* 1900
figs, *Ephestia kuehniella* 1899
foods, *Ptinus tectus* 1899
ginger, *Ahasverus advena* 1899
guavas, *Attagenus unicolor* 1900
melons, *Dacus xanthodes* 1899
oranges, *Caliothrips fasciatus* 1900
pallets, *Tridomyrmex anceps* 1899
Vicia faba, Bruchus pisorum 1899
milk products, *Salmonella* 562
timbers
Coptotermes acinaciformis 1764
Coptotermes frenchi 1764
Kalotermes brouni 213
Platypus 1775
Stolotermes inopinus 1763
Stolotermes ruficeps 1763
wood preservation
marketing 1783
standards 1190
Nickel chloride, *Litchi, Geotrichum candidum* 1620
Nickel sulphate, *Litchi, Geotrichum candidum* 1620
Nicotine, control, *Lasioderma serricorne* 1934
Nigeria
beef, *Staphylococcus* 161
cereals, mycotoxins 1546
cowpeas, *Callosobruchus maculatus* 82
food products, *Staphylococcus aureus* 10
infant formulae, *Staphylococcus* 1673
maize
aflatoxins 938
insect pests 1521
ochratoxins 938
zearalenone 938

Nigeria *cont.*
malt
Aspergillus fumigatus 1518
Aspergillus versicolor 1518
mangoes, *Dothiorella dominicana* 16
oranges, beverages, microbial flora 1
paints
Cladosporium 1821
Phialophora verrucosa 1821
pineapples, beverages, microbial flora 102
Nisin
Bacillus licheniformis, preservatives, foods 28
bacon, *Clostridium botulinum* 554
Lactobacillus, resistance 26
meat products
Lactobacillus 26
microbial count 1070
milk, psychrotrophic bacteria 1690
Nitrification
activated sludge 1976
oxidation ditches 1981
Nitrilotriacetic acid, biodegradation 2021
Nitrogen, control, *Tribolium confusum* 1938
Nitrogen fixation
cellulose, utilization, *Azospirillum brasilense* 1339
straw, utilization, *Azospirillum brasilense* 1339-1340
***P*-nitrophenol,** biodegradation, *Pseudomonas* 762
1-Nitropyrene, detoxification, *Cunninghamella elegans* 355
***N*-nitrosodimethylamine,** biodegradation 1330
Nocardia, rubber, biodegradation 813
Nocardia erythropolis, phthalic acid ester biodegradation, activated sludge 132
Nonvolatile residue, biodegradation, *Pseudomonas cepacia* 2060
North America, Polyporaceae, books 1406
Nut products, aflatoxins, United Kingdom 972
Nuts
aflatoxins
Mexico 484
United Kingdom 972
insecticides, losses 751
mycotoxins 483
pest control, ethyl formate 744
Nylon manufacturing wastes, biodegradation, *Pseudomonas cepacia* 2060
Nystatin, pears, *Botryodiplodia theobromae* 1008
Oats
Fusarium 448
insects, estimation 1517
Rhyzopertha dominica, estimation 1517
Sitophilus oryzae, estimation 1517
stored products pests, methoprene 151
Ocean thermal energy conversion plant, biofouling, control 1836
Oceans, sewage sludge, disposal 1987
Ochlandra travancorica, insect pests, control 1729
Odontotermes, timbers, susceptibility 217
Odour abatement, filtration, bacteria 311
Oerskovia, waste water treatment, food industry 1302
Offal
bacterial count 8
microbial flora 1665
Oil-borne compounds, wood preservatives 671-672, 1411, 1421, 1802
Oil-borne solvents, wood preservatives 141
Oilfield water injection systems, bacteria, biocides 1825
Oils
biodegradation, bacteria 798
production, whey 1370
recovery
Bacillus licheniformis, surfactants 356
surfactants, reviews 354
Oligochaeta, sewage sludge, stabilization 1961
Olive marc, land application, agronomy 1989

ive oil
aflatoxins 485
control, *Callosobruchus maculatus* 81
ochratoxins 485
ives
aflatoxins 485
Aspergillus 485
ochratoxins 485
Penicillium 485
pitrichum, rubber, enzymes 1214
nmatoiulus moreletii
distribution
Australia 257
Azores 257
Madeira 257
Portugal 257
nions
Botrytis 133, 144
pest control, storage structures 12
postharvest treatment, maleic hydrazide 1648
Pseudomonas aeruginosa, Papua New Guinea 1039
storage, quality, cultural methods 529
storage decay 136, 1040
ospora lactis, taxonomy 1844
range juice, aflatoxins, bioassays 889
range peel
pectinase, production 2034
proteins, production 2034
utilization, *Aureobasidium pullulans* 2034
ranges
beverages, microbial flora, Nigeria 102
Caliothrips fasciatus, New Zealand, interceptions 1900
Penicillium, thiabendazole 514
Penicillium digitatum
sodium 2-phenylphenolate 1023-1024
thiabendazole 1023-1024
storage
biochemistry 1019
China 1619
controlled atmosphere storage 513
storage decay, 2-phenylphenol 523
Organic acid recovery system,
Trichosporon beigelii, biofouling 1835
Organic solvents, wood preservatives 1189
Organochlorine compounds
biodegradation
Acinetobacter 2012
Phanerochaete chrysosporium 333
Organoiodine compounds, wood preservatives 1812
Organotin compounds
algicides 683
building materials, algae 683
fungicides 756
leaching, bacteria 1229
paints, biofouling, United Kingdom 692
paper, fungi 756
wood preservatives 220, 1812
Oryzaephilus, physiology, aggregation pheromones 1879
Oryzaephilus mercator
cereals
China 1509
cold resistance 752
identification 260
oats, methoprene 1516
physiology, aggregation pheromones 1878
rapeseed 481
Oryzaephilus surinamensis
cereals, Canada 1491
control
laser radiation 1887
methyl chloroform 736
precocenes 1276
sorptive coatings 1930
2-tridecanone 1865
feeds, German Federal Republic 908
military rations 855
monitoring, traps 1890
oats, methoprene 1516
physiology
aggregation pheromones 1878
respiratory system 1881
population dynamics 1251
Oryzaephilus surinamensis *cont.*
raisins, controlled atmospheres 1929
rapeseed 481
resistance
fenitrothion 287
insecticides 279
Australia 1882
malathion 280, 1277
permethrin 1277
phosphine 272, 292
pirimiphos-methyl 280
rice, biology, reproduction 1533
sesame
attapulgite 974
bromophos 974
etrimfos 974
malathion 974
methacrifos 974
pirimiphos-methyl 974
pyrocon 974
sorghum, insecticides 1567
stored products
Japan 1891
United Kingdom 1890
traps 276
wheat
chlorpyrifos-methyl 921
etrimfos 921
fenitrothion 921
heat treatment 466
malathion 921
methacrifos 921
pirimiphos-methyl 921
refrigeration 1554
sorptive silica dusts 1558
Turkey 471
Oxidation ditches
denitrification 1981
nitrification 1981
Oxirane, control, *Rhyzopertha dominica* 1254
Oxydong, fungicide tolerance, *Alternaria mali* 300
Oysters
bacteria 147
Clostridium perfringens, United Kingdom 542
Escherichia coli, United Kingdom 542
Klebsiella pneumoniae, health hazards 1663
Streptococcus faecalis, United Kingdom 542
Ozone, apples, storage 1002
Pachnocybe ferruginea
poles 1850
taxonomy 1850
Pachysolen tannophilus
cellulosic wastes, utilization 2018
D-xylose
utilization 1332
biodegradation 2003
Packaging
Bacillus subtilis, decontamination 685
decontamination, ultraviolet radiation 685
insect pests 837
lemons, *Penicillium digitatum* 1621
tangors, *Penicillium digitatum* 1621
Packaging materials, groundnuts, fungi 93
Packhouses
Botrytis, pome fruits 507
Mucor piriformis, pome fruits 507
Penicillium, pome fruits 507
vegetables, storage decay 499
Paecilomyces
agricultural wastes, utilization 2002
polyvinyl chloride 1217
Paecilomyces variotii, control, polyoxin 1843
Paintings, fungi, India 1830
Paints
biodeterioration, bacteria 1226
Cladosporium
Nigeria 1821
walls 1821
fungicides, testing 1822
organotin compounds, biofouling, United Kingdom 692
Phialophora verrucosa
Nigeria 1821
walls 1821
Paints *cont.*
Trentepohlia odorata, control 695
Pakistan
aflatoxins, maize 64
composting, refuse 326
spices, microbial count 988
Pallets, *Tridomyrmex anceps,* New Zealand, interceptions 1899
Palm kernel oil, fungi, fatty acids 981
Palms, wines, preservation 98
Palorus subdepressus, cereals, Canada 1491
Panels, preservation 673
Panneer, quality 1081
Panonychus ulmi, apples, carbon dioxide 110
Panus tigrinus, potato waste, utilization 1313
Paper
foxing, *Aspergillus* 233
fungi
organotin compounds 756
testing, standards 1211
Papua New Guinea, onions, *Pseudomonas aeruginosa* 1039
Paraguay, timbers, decay, resistance 1744
Parathion-methyl, *Ochlandra travancorica,* insect pests 1729
Parchment
Bacillus 628
bacteria, proteolysis 629
Parlatoria pergrandii, mandarins, control 1017
Particleboards
Chaetomium globosum, adhesives 1202
Coniophora puteana
adhesives 1202
F compounds 1749
furcarbanil 1749
Coriolus versicolor
adhesives 1202
electron microscopy 201
F compounds 1749
furcarbanil 1749
fungi 1176
Polyporus palustris, electron microscopy 201
Poria placenta
F compounds 1749
furcarbanil 1749
preservation 673
testing 1195
wood preservation 662
methodology 226
Passion fruits, storage, plastic film 994
Pasta, pest control, books 837
Pastes, shrimps, aflatoxins, Singapore 37
Pasteurization, metalworking fluids, bacteria 1818
Pasteurized milk, *Salmonella,* heat treatment 1718
Patents
dried whey, utilization, baking 775
whey
fermentation
Lactobacillus casei 810
Propionibacterium shermanii 810
Pawpaws
Ceratitis capitata
ethylene dibromide 1623
quarantine 1623
Dacus cucurbitae
ethylene dibromide 1623
quarantine 1623
Dacus dorsalis
ethylene dibromide 1623
quarantine 1623
fungi, gamma radiation 1618
Trichothecium roseum, biochemistry 1634
PCP
biodegradation
bacteria 2024
Flavobacterium 2024
fungicidal properties 1942
assays 1209
sewage sludge, biodegradation 1355
timbers
Gloeophyllum trabeum 1793
stain fungi 1171

PCP *cont.*
wood preservatives 663, 1196, 1414, 1773, 1793, 1795
PCP-Na
canes and rattans, stain fungi 1166
wood preservatives 663, 1166, 1199, 1206, 1804, 1814
Peaches
Botrytis cinerea 1011, 1616
Ceratitis capitata, heat treatment 1010
Euzophora bigella, United Kingdom 1614
Monilinia fructicola, biological control 1617
Monilinia laxa 1616
Penicillium expansum 1011
postharvest decay, control 1598
postharvest treatment 511, 1009
Rhizopus stolonifer 1011, 1613, 1616
actidione 115
boric acid 115
storage, quality, calcium 512
Pearl millet
Ephestia cautella, control 943
Rhyzopertha dominica, varietal susceptibility 945
Sitophilus oryzae, varietal susceptibility 945
Sitotroga cerealella, varietal susceptibility 945
Trogoderma granarium, etrimfos 1536
Pears
Botryodiplodia theobromae, nystatin 1008
Botrytis, fungicides 1605
Botrytis cinerea 1610
fungi, control 112
Mucor piriformis 1610
Penicillium, fungicides 1605
Penicillium expansum 1610
cold storage 1604
controlled atmosphere storage 1604
postharvest decay, control 1598
storage
cold storage 1006
controlled atmosphere storage 109, 1006
diseases, France 1003
quality 1601
growth regulators 501
Peas
Callosobruchus chinensis 78
Callosobruchus maculatus 78
Sitophilus oryzae
development 473
phytotoxins 1574
Peat, pressing, waste water treatment 366
Pecans
mycotoxins 91, 483
Penicillium 91
storage, cold storage 90
toxinogenic fungi 977
Pectinase, production, orange peel 2034
Pectinophora gossypiella, cotton, control, machinery 1730
Penicillium
agricultural wastes, utilization 1314
canes and rattans 1162
cheeses 193
control, ammonia 1941
feeds 420, 907
USSR 911
foods 48, 420
guavas, coatings 1627
identification, books 386
lemons, control 519
maize, storage decay 462
milk products, Egypt 1094
olives 485
oranges, thiabendazole 514
packhouses, pome fruits 507
pears, fungicides 1605
pecans 91
physiology, sodium chloride 706
polyvinyl chloride 1217
proteins, production 1314
resistance, benomyl 507
sorghum, Egypt 72
spices 490
taxonomy 703
books 1404
Penicillium *cont.*
Terminalia 122
tomatoes, ethylene 1641
Penicillium citrinum
polyurethanes 682
biodeterioration 234
polyvinyl chloride 1217
Penicillium corymbiferum, garlic, Chile 1650
Penicillium cyclopium
apples, controlled atmosphere storage 1007
soyabeans, captan 475
Penicillium digitatum
citrus fruits 1022
control 120, 521
wax coatings 1027
grapefruits, ethylene 121
isolation, culture media 866
lemons
fungicides 1621
packaging 1621
waxes 1621
oranges
sodium 2-phenylphenolate 1023-1024
thiabendazole 1023-1024
tangors
fungicides 1621
packaging 1621
waxes 1621
tolerances, potassium sorbate 1028
Penicillium expansum
apples
calcium 114
control 505
controlled atmosphere storage 114, 1007
fungicides 502, 509
control
heat treatment 506
sodium 2-phenylphenolate 506
enzymes, cellulases 1849
fruits, physiology 106
nectarines 1011
patulin, apple juice 493
peaches 1011
pears 1610
cold storage 1604
controlled atmosphere storage 1604
pome fruits, fungicide tolerance 113
Penicillium frequentans, pomegranates, India 1626
Penicillium granulatum, apples, controlled atmosphere storage 1007
Penicillium italicum
citrus fruits 1022
wax coatings 1027
control, plant extracts 1947
tolerances, potassium sorbate 1028
Penicillium jenseni, wheat 958
Penicillium mali, apples, controlled atmosphere storage 1007
Penicillium pittii, taxonomy 254
Penicillium rademirici, taxonomy 254
Penicillium roquefortii
cheeses 192
1,3-pentadiene 1150
Penicillium samsonii, taxonomy 254
Penicillium sclerotigenum, yams, enzymes 135
Penicillium verruculosum
proteins, production 1319
straw, upgrading 1319
Peniophora
timbers
wood anatomy 642
wood chemistry 642
Peniophora cinerea, timbers 633
Peniophora sanguinea, timbers, wood chemistry 1172
Peniophora violaceo-livida, timbers 633
Pentachlorophenyl laurate, wood preservatives 1812
Perenniporia tephropora, timbers, creosotes 1750
Permethrin
control
insect pests 737
Lasioderma serricorne 1934
Tribolium castaneum 290
Permethrin *cont.*
logs 225
maize
insect pests 1521
pest control 63
Prostephanus truncatus 936, 15
Sitophilus zeamais 60
Ochlandra travancorica, insect pest 1729
resistance
Oryzaephilus surinamensis 127
Sitophilus oryzae 1861
sorghum, insect pests 71
stored products, pest control 63
timbers
insect pests 658
Isoptera 1765
wood borers 1179
wood preservatives 662
wool textiles, insect pests 1816
Peru, potatoes, pest control 137
Pest control
aircraft 824
cereals
Australia 750
Austria 293
crops, losses, tropics 1
food industry
conferences 1396
reviews 1281
foods, conferences 824
grain stores, Canada 753
India 1280
maize, permethrin 63
potatoes
Peru 137
pheromones 139, 283
protective atmospheres 720
stored products
permethrin 63
timing 296
timbers, quarantine 212
Pest management, books, Scolytidae 834
Pestalotia
fruits, physiology 106
guavas, coatings 1627
Pests, books, field crops, United Kingdo 836
Pests management, foods, books 1399
Petroleum
Amorphotheca resinae 1392
biodegradation, bacteria 1349
waste water treatment, reviews 2055
Petroleum hydrocarbons
effluents
Aeromonas 357
Azotobacter 357
Bacillus 357
Pseudomonas 357
pollution, treatment, microorganisms 1959
Petroleum oils
timbers, *Coniophora puteana* 1200
wood preservatives 1200
Phallus impudicus
Fagus 649-650
physiology, water relations 1847
timbers 206
Phanerochaete chrysosporium
bark, utilization 315
cellulose, biodegradation 631
DDT, biodegradation 333
dioxin, biodegradation 333
enzymes 197, 1241
lignin, biodegradation 705
ligninase 250, 373, 1734, 2067
ligninases 251
genetics 1848
lignin
biodegradation 197, 347, 376, 1385
chemistry 1159
enzymes 1734
ligninase 371
ligninolytic microorganisms 1841
organochlorine compounds, biodegradation 333
recombination, genes 376
timbers 633
Phanerochaete sanguinea, timbers, wood chemistry 1172

anerochaete velutina
Fagus 649-650
wood, growth 1173
armaceutical industry, waste water treatment, reviews 2039
armaceuticals, microbial contamination, testing 1820
aseolus coccineus, *Acanthoscelides obtectus* 145
aseolus vulgaris
Acanthoscelides obtectus 145
cold storage 146
fumigants 146
heat treatment 146
seed penetration 79
tricalcium phosphate 1566
Callosobruchus chinensis, tricalcium phosphate 1566
Callosobruchus maculatus
oviposition 721
tricalcium phosphate 1566
storage, cold storage 1596
ellinus igniarius, timbers, strength of wood 204
ellinus noxius, *Hevea brasiliensis,* lignin, degradation 1758
ellinus pini
cellulose, biodegradation 631
control, wood preservatives 1777
ellinus tremulae, wood, Canada 634
henolic compounds
timbers, *Gloeophyllum trabeum* 1793
wood preservatives 1793
henols
biodegradation
activated sludge 2028
Alcaligenes faecalis 1357
bacteria, kinetics 2029
Candida tropicalis 2026-2027
Pseudomonas alcaligens 1357
Pseudomonas putida 800
waste water treatment, ligninase 371
henothrin
maize, *Prostephanus truncatus* 936
resistance, *Sitophilus oryzae* 1861
sorghum, insect pests 71
henylmercuric acetate, murals, *Beauveria alba* 1829
-Phenylphenol
lemons, storage decay 523
oranges, storage decay 523
heromones
groundnuts, *Ephestia cautella* 976
potatoes, pest control 139, 283
hialocephala dimorphospora, poles, strength 644
hialophora
detoxification, tributyltin oxide 1948
poles, strength 644
timbers, reviews 202
hialophora fastigiata, poles, strength 644
hialophora heteromorpha, poles, strength 644
hialophora hoffmannii, detoxification, tributyltin oxide 1948
hialophora japonica, timbers 255
hialophora malorum
control
heat treatment 506
sodium 2-phenylphenolate 506
detoxification, tributyltin oxide 1948
hialophora verrucosa
paints
Nigeria 1821
walls 1821
Philippines
cereals, storage 1495
foods, aflatoxins 902
Phlebia brevispora
ligninolytic microorganisms 1841
poles, physiology 1752
Phlebia tremellosus, timbers, decay 1761
Phlebiopsis gigantea
control, PCP 1209
timbers 1209
Pholiota mutabilis, ligninolytic microorganisms 1841
Phoma, pomegranates, India 1626
Phoma eupyrena, potatoes, controlled atmosphere storage 1046
Phoma exigua* var. *exigua, potatoes, controlled atmosphere storage 1046
Phoma exigua* var. *foveata
potatoes
controlled atmosphere storage 1046
epidemiology 1045
Phoma punicae, pomegranates, India 1626
Phomopsis, pomegranates, India 1626
Phomopsis psidii, guavas, biochemistry 128
Phosphates
control, *Clostridium botulinum* 1839
meat products, *Clostridium botulinum,* reviews 1668
Phosphine
cereals, insects 748
control
Callosobruchus chinensis 281
Ephestia cautella 1256
insect pests 1290
insects 742
Lasioderma serricorne 1923
Rhyzopertha dominica 1254
Sitophilus granarius 1273
stored products pests 1924
Tribolium confusum 1938
Trogoderma granarium 271
Trogoderma variabile 1262
fumigants 743
historical buildings, *Hylotrupes bajulus* 688
maize, insects 747
resistance
insect pests 272, 292
insects 719
stored products pests, Australia 1882
Tribolium castaneum 1255, 1933
Trogoderma granarium 1886
rice, insect pests 66
wheat, *Sitophilus oryzae* 1559
Phosphorus
release, bacteria 804
removal
waste water treatment
Acinetobacter 2030
activated sludge 2030
Phoxim
control, *Ephestia cautella* 1284
timbers, insect pests 658
Phragmites australis, biodeterioration, thatch 242
Phthalic acid esters, biodegradation, *Nocardia erythropolis,* activated sludge 1327
Phthorimaea operculella
potatoes
control 140, 1042
oviposition deterrents 1651
resistance 138
varietal susceptibility 533
Phyllanthus emblica, *Alternaria alternata* 119
Physical control, feeds, mites 1473
Phytophthora, guavas, coatings 1627
Phytophthora cryptogea, apples, German Democratic Republic 1609
Phytophthora porri, Chinese cabbages 537
Phytosciara zingiberis, ginger, Japan 987
Picea abies
Sporotrichum pulverulentum, wood anatomy 636
Xyloterus lineatus, slaked lime 221
Pichia stipitis, xylan, biodegradation 2019
Pig slurry, treatment, *Scenedesmus* 330
Pigments, biodeterioration, bacteria 1226
Pigs, manures, digesters, bacteria 1322
Piles
preservation 1801
fumigants 1796
wood preservation 222
Pineapples
Acetobacter liquefaciens, symptoms 1635
beverages, microbial flora, Nigeria 102
Ceratocystis paradoxa 124
Curvularia verruculosa 124
Pinus
fumigation, HCN 239
fungi, wood preservation 205
Pinus cont.
Serpula lacrimans, laboratory tests 1754
Pinus sylvestris
Cytospora pini, wood cement 638
decay fungi, logging, seasonal variation 640
Tomicus piniperda, control 218
Xyloterus lineatus, slaked lime 221
Pinus taeda
Peniophora
wood anatomy 642
wood chemistry 642
Piperonyl butoxide
control, *Prostephanus truncatus* 1939
Vigna radiata, Callosobruchus chinensis 1569
Pipes, bacteria, USSR 1225
Pirimiphos-methyl
barley, *Lepidoglyphus destructor* 933
cereals, *Sitophilus oryzae* 285
control
Ephestia cautella 1264, 1284
insect pests 737, 1282
flour mills 1243
Lasioderma serricorne 1934
cowpeas, *Callosobruchus maculatus* 1569
dried fish
Dermestes maculatus 1056
Necrobia rufipes 1056
maize
insect pests 1521
Prostephanus truncatus 936, 1520
Sitophilus zeamais 60
raisins, insects 1036
resistance
Oryzaephilus surinamensis 279-280
Rhyzopertha dominica 1901
Sitophilus zeamais 1901
rye, *Cryptolestes ferrugineus* 1494
sesame
Oryzaephilus surinamensis 974
Tribolium castaneum 974
sorghum, insect pests 71
triticale, *Cryptolestes ferrugineus* 1494
wheat
Cryptolestes ferrugineus 1494
Oryzaephilus surinamensis 921
Sitophilus granarius 921
Tribolium castaneum 921
Pityogenes chalcographus, timbers, lime (mineral) 1772
Planococcus dioscoreae, yams, Ivory Coast 1647
Planofix, apples, *Alternaria alternata* 1612
Planotortrix excessana
apples
chlorpyrifos 1607
controlled atmosphere storage 1608
Plant extracts
antifeedants, stored products pests 731
antifungal properties 298
beans (*Phaseolus*), *Acanthoscelides obtectus* 738
cereals
Callosobruchus chinensis 1285
Sitophilus oryzae 1285
Trogoderma granarium 1285
control
Aspergillus 1949
fungi 1240
Rhyzopertha dominica 1936
Sitotroga cerealella 1869
stored products pests 1288, 1863
Tribolium castaneum 1898
groundnuts, storage decay 1949
maize, *Sitophilus oryzae* 1937
potatoes, *Phthorimaea operculella* 1651
rice
Rhyzopertha dominica 939
Sitophilus oryzae 939
Sitotroga cerealella 939
sterilization, *Tribolium castaneum* 1883
wheat
Rhyzopertha dominica 1916
Sitophilus oryzae 1916
Sitotroga cerealella 1283

Plant oils
mung beans
Callosobruchus chinensis 961
Callosobruchus maculatus 961
Plant residues
single cell proteins, production 2000
utilization
Cladosporium herbarum 2000
Cochliobolus lunatus 2000
Plants, municipal refuse disposal, land application 1312
Plastic film
Capsicum annuum, storage decay 528
citrus fruits, decay 1621
fungi 1217
packing
Averrhoa carambola 1018
passion fruits 994
tomatoes 994
tomatoes, storage decay 527
Plastics
biodeterioration 1216
timbers, Isoptera 1765
Platypodidae
logs, control 225
timbers
Japan, interceptions 656, 1248
Korea Republic, interceptions 215
Platypus, timbers, New Zealand 1775
Pleospora herbarum
documents 1212
enzymes, cellulases 1212
Pleurotus, straw, recycling 1341
Pleurotus ostreatus
enzymes 1238, 1388
ligninolytic microorganisms 1841
Pleurotus sajor-caju
cellulolytic microorganisms 372
enzymes, cellulase 372
Plodia interpunctella
almonds, controlled atmospheres 1929
cereals, microbial pesticides 1493
control
insecticides 1243
sorptive coatings 1930
sterile insect release 1926
2-tridecanone 1865
development 707
dried fruit, biology 1872
feeds, German Federal Republic 908
flour mills, Cyprus 1243
groundnuts, ecology 1586
military rations 855
physiology, enzymes 1858
raisins
controlled atmospheres 1929
Cyprus 1036
insecticides 1036
microbial pesticides 1036
resistance, insecticides, Australia 1882
stored products
Australia, New South Wales 1871
Japan 1891
traps 263
wheat, control 1547
Plums
Botrytis cinerea 1616
Monilinia laxa 1616
prestorage treatment, iodine 510
Rhizopus stolonifer 1616
Plywood
Coriolus versicolor, laboratory tests 639
Gloeophyllum trabeum, laboratory tests 639
Lentinus cyathiformis, laboratory tests 639
preservation 673
Stereum, laboratory tests 639
wood preservation 662
methodology 226
Poland
Buprestis aurulenta, distribution 273
Buprestis haemorrhoidalis, distribution 273
Buprestis rustica, distribution 273
cereals, insect pests 454
Chalcophora mariana, distribution 273
drug formulations, microbial contamination 235-236
kidneys as food, ochratoxins 1074
Poland *cont.*
milk
bacterial count 1126
coliform bacteria 1129
mycotoxins 613
stored products, *Tyrophagus longior* 724
timbers, wood borers 659
wheat
Fusarium 472
mycotoxins 472
Poles
Coriolus versicolor, physiology 1752
decay
preservation 637
Sweden 1163
decay fungi, strength 644
fungi
methyl isothiocyanate 1203
USA 643
Gloeophyllum saepiarium, physiology 1752
Gloeophyllum trabeum, physiology 1752
Isoptera
Guam 1773
wood preservation 1773
Lentinus lepideus, physiology 1752
Pachnocybe ferruginea 1850
Phlebia brevispora, physiology 1752
Poria, physiology 1752
Poria carbonica, physiology 1752
Poria placenta
physiology 1752
preservation 1808
Poria radiculosa, physiology 1752
Poria xantha, physiology 1752
preservation
chloropicrin 1808
creosotes 1784
fumigants 1796
Latin America 1785
methyl isocyanate 1808
polyethylene 1808
pressure treatments 671-672
standards, Taiwan 1805
Radulum concentricum 210
wood preservation 1163
Pollutants, chemical industries, bacteria, genetic engineering 328
Pollution
control, microorganisms 1292
petroleum hydrocarbons, treatment, microorganisms 1959
Polychlorinated biphenyls
biodegradation
Acinetobacter, genetic engineering 359
Alcaligenes, genetic engineering 359
assays 1329
Polydong, fungicide tolerance, *Alternaria mali* 300
Polyesters, bacteria, adhesion 1817
Polyethylene, preservation, poles 1808
Polyethylene film, storage, lettuces 1655
Polygalacturonase, production, *Cryptococcus albidus* 253
Polymers
fungi 1215
resistance, microorganisms 681
Polyoxin
control, fungi 1843
fungicide tolerance, *Alternaria mali* 300
Polyphosphates, chicken meat, *Salmonella typhimurium* 1076
Polyplastron multivesiculatum
cellulolytic microorganisms 1160
cellulose, biodegradation 1160
fish 1058
seafoods 1058
Polyporaceae, North America, books 1406
Polyporus adustus, *Fagus* 648, 650
Polyporus meliae, timbers, resistance 647
Polyporus palustris
enzymes, cellulases 700
particleboards, electron microscopy 201
timbers
laboratory tests 1748
resistance 647
Polyporus squamosus, taxonomy 1844
Polyurethanes
biodeterioration, *Penicillium citrinu* 234
fungi 682
Polyvinyl chloride, fungi 1217
Pome fruits
bibliographies 111
Botrytis, packhouses 507
Mucor piriformis, packhouses 507
Penicillium, packhouses 507
Penicillium expansum, fungicide tolerance 113
Pomegranates, postharvest decay, India 1626
Ponds, waste water treatment, reviews 1980
Pongamia glabra
mung beans, *Callosobruchus chinens* 922
sorghum, *Sitophilus oryzae* 922
Pongamia pinnata, potatoes, *Phthorima operculella* 1651
Poria, poles, physiology 1752
Poria carbonica
poles, physiology 1752
timbers
chloropicrin 223
methyl isothiocyanate 677
Poria medulla-panis
lignin 209
ligninolytic microorganisms 209
timbers, wood chemistry 200
Poria monticola
timbers
acetylation 1780
wood properties 203
Poria placenta
control, PCP 1209
particleboards
F compounds 1749
furcarbanil 1749
poles
physiology 1752
preservation 1808
timbers
hydrogen peroxide 1175
resistance 1744
wood, wood anatomy 1746
Poria radiculosa, poles, physiology 1752
Poria xantha, poles, physiology 1752
Pork
bacteria, culture media 555
bacterial count, techniques 159
Campylobacter coli 549
Campylobacter jejuni, storage 553
microbial count, chilling 548
psychrotrophic bacteria, sodium hypochlorite 158
Yersinia enterocolitica 857
inhibition, microflora 1669
virulence 697
Portugal, *Ommatoiulus moreletii,* distribution 257
Post pasteurization contamination, milk 189
Postharvest decay
asparagus, methyl bromide 1051
guavas, coatings 1627
pomegranates, India 1626
potatoes, Egypt 142
vegetables, India 498
Postharvest losses
Caribbean 500
rice 465
Postharvest storage decay, fruits, reviews 126
Postharvest systems, education, India 1423
Posts
bacteria, Cu-Cr-As 1739
decay, Cu-Cr-As 1210
wood preservation 1191, 1811
Potassium chloride, fish, *Clostridium botulinum* 541
Potassium nitrite, milk, preservation 1693
Potassium sorbate
apple juice, aflatoxins 895
chicken meat, *Salmonella* 558
control
Geotrichum candidum 522
mycotoxins 33

ıssium sorbate *cont.*
lates, preservation 1021
emons, *Geotrichum candidum* 522
nilk, psychrotrophic bacteria 188
Penicillium digitatum, tolerances 1028
Penicillium italicum, tolerances 1028
ıto factory effluent, water pollution, rivers 302
ıto starch
ermentation, *Saccharomycopsis fibuligera* 1316
ıtilization
Candida utilis 1317
Saccharomyces diastaticus 1317
Saccharomycopsis fibuligera 1317
Schwanniomyces alluvius 1317
ato waste
anaerobic treatment 2035
land application 1308
proteins, production 1313
utilization, basidiomycetes 1313
atoes
arthropod pests, India 1041
bacteria
captan 1043
carboxin 1043
thiabendazole 1043
Cercospora solanicola, India 1652
Clostridium botulinum, preservatives 534
Erwinia carotovora subsp. *atroseptica* 141
varietal reactions 1654
Erwinia carotovora subsp. *carotovora*
enzymes 1653
grading 1044
Fusarium
captan 1043
carboxin 1043
thiabendazole 1043
Fusarium oxysporum, epidemiology 1045
pest control 1041
Peru 137
pheromones 139, 283
storage structures 12
Phoma exigua var. *foveata,* epidemiology 1045
Phthorimaea operculella
control 140, 1042
oviposition deterrents 1651
resistance 138
varietal susceptibility 533
postharvest decay, Egypt 142
storage decay, controlled atmosphere storage 1046
Symmetrischema plaesiosema, control 1042
oultry
effluents, water pollution, rivers 302
Salmonella typhimurium, adhesion 1670
oultry industry, wastes, treatment, reviews 2036
oultry manure, single cell proteins, *Rhizopus chinensis,* cassava 331
oultry meat
bacterial count, Japan 170
coliform bacteria, enumeration 863
Escherichia coli
Egypt 168
enumeration 863
ochratoxins 1080
Salmonella, Egypt 168
Salmonella typhimurium, freezing techniques 1077
sampling, automation 172
Staphylococcus aureus, Egypt 168
Streptococcus faecalis, Egypt 168
ower generation, waste waters, shrimp culture 1384
ower industry, waste water treatment, reviews 2064
rawns
bacteria, trimethylamine oxide, reduction 540
bacterial count
cold storage 1060
irradiation 1060
recocenes, control, *Oryzaephilus surinamensis* 1276
Prepared foods
microbial count, temperature 9
microbial flora, temperature 70
Preservation
infant formulae, lactoperoxidase system 1121
marine structures, marine borers 220
palms, wines 98
Preservatives
Clostridium botulinum, bacon 157
contact lens formulations, evaluation 1219
foods, *Bacillus licheniformis,* nisin 28
wines
propionic acid 98
sodium benzoate 98
sodium metabisulphite 98
Pressure treatments
poles, preservation 671-672
wood preservation 1189
Processed foods, bacterial count 8
Prochloraz
apples
Botrytis cinerea 509
Penicillium expansum 509
mangoes, fungi 1014
melons, storage decay 1031
Procymidone, pears, storage, diseases 1605
Propineb, ginger, fungi 1593
Propionibacterium acidi, whey, fermentation 775
Propionibacterium rubrum
barley, preservation 2041
whey, utilization 2041
Propionibacterium shermanii
citrus, wastes, fermentation 1360
molasses, fermentation 2033
whey
fermentation 2033
patents 810
Propionic acid
bananas, *Aspergillus niger* 1633
preservation, beer 1595
preservatives, wines 98
production, whey 2041
Propionic acid bacteria
molasses, fermentation 2033
whey, fermentation 2033
Propoxur
potatoes, *Phthorimaea operculella* 140
timbers, insect pests 658
Prostephanus truncatus
cassava
control 1892
Kenya 1892
Tanzania 530, 1892
control
cyfluthrin 1939
deltamethrin 1939
laser radiation 1887
piperonyl butoxide 1939
identification 1875
maize
control 936, 1520, 1892
Kenya 1520, 1892
storage losses, Tanzania 63
Tanzania 1892
Togo 62
stored products, Africa, reviews 1860
Protective atmospheres
control
Acarus siro 725
insects 741
pest control 720, 1279
Proteins
production
Absidia 1315
Aspergillus 1314
cellulosic wastes 2017
Chaetomium cellulolyticum 1319
Chrysosporium 1315
Mucor 1315
orange peel 2034
Penicillium 1314
Penicillium verruculosum 1319
potato waste 1313
Rhizopus 1314
rice straw 2001
Thielavia 1315
whey 1363, 1996, 2050
Proteus
milk 610
milk products, Egypt 1095
shrimps, indoles 151
Providencia, milk products, Egypt 1095
Pseudobacterium boreale, metals, seawater 1222
Pseudomonas
beef 162
bacteriophages 1068
cod 149
cooling systems, fouling 1230
culture media 398
detergents, biodegradation 799
2,4-dichlorophenol, biodegradation 762
ewe milk, Bulgaria 1697
meat 165
metalworking fluids 1819
milk 1118
molasses, residues, utilization 1380
naphthalene, biodegradation 797
p-nitrophenol, biodegradation 762
organotin compounds, leaching 1229
petroleum hydrocarbons, effluents 357
sodium dodecyltriethoxy sulphate, biodegradation 1352
Pseudomonas acidovorans, α-methyl styrene, biodegradation 1345
Pseudomonas aeruginosa
beef
acetic acid 1066
formic acid 1066
culture media 4
DDT, biodegradation 1356
disinfectants 299
hydrocarbons, biodegradation 1348
onions, Papua New Guinea 1039
resistance
biocides 1943
formaldehyde 1944-1945
triazines 297
Pseudomonas alcaligens, phenols, biodegradation 1357
Pseudomonas atlantica, biofilms, metals 693
Pseudomonas cepacia, nylon manufacturing wastes, biodegradation 2060
Pseudomonas ceracia, 2-chlorobenzoic acid, utilization 780
Pseudomonas diminuta, chlorinated anilines, biodegradation 1325-1326
Pseudomonas fluorescens
formaldehyde, biodegradation 1354
milk 607
naphthalene, biodegradation 1350
proteinases, differentiation 244
susceptibility, butylated hydroxyanisole 1950
Pseudomonas fragi
stainless steel, adhesion 237
susceptibility, butylated hydroxyanisole 1950
Pseudomonas gladioli* pv. *alliicola, onions 136
Pseudomonas lundensis
description 1234
meat 1234
Pseudomonas putida
4-chlorobenzoic acid, utilization 781
2,4-dichlorobenzoic acid, utilization 781
germanium, recovery 2004, 2062
naphthalene, biodegradation 1350
phenols, biodegradation 800
resistance
biocides, genetics 1946
formaldehyde 1945
Pseudotsuga, Radulum concentricum 210
Pseudotsuga menziesii, Poria carbonica, methyl isothiocyanate 677
Psilosciara flammulinae, ginger, Japan 987
Psocidae, cereals, Canada 1491
Psocoptera
food industry, United Kingdom 1258
foods, United Kingdom 1259-1260
Psophocarpus tetragonolobus, fungi, control 1580
Psychrotrophic bacteria
culture media 398
dairy factories
cooling water 238

Psychrotrophic bacteria *cont.*
dairy factories *cont.*
pasteurizers 189
enzymes
lipase, reviews 1837
proteinases, reviews 1232
ewe milk
Bulgaria 1697
cold storage 1698
foods, enzymes, proteases 696
milk
amino acids 1690
cold storage 588, 1694
detection 1102
Egypt 1127
gelation 1103
hydrogen peroxide 188
nisin 1690
oxygen, tension 1708
potassium sorbate 188
proteinases 1141, 1232
sensory evaluation 1677
milk products
enzymes 1087
sensory evaluation 1677
pork, sodium hypochlorite 158
proteinases, differentiation 244
Pterotermes occidentis, structural wood 216
Ptinus tectus, foods, New Zealand, interceptions 1899
Pulp and paper industry, effluents, treatment, reviews 2057
Pulp mill effluent
anaerobic digestion 2058
Azotobacter, waste water treatment 308
single cell proteins, production 1381
utilization, *Chaetomium cellulolyticum* 1381
D-xylose, fermentation 365
Pulping materials
preservation 1814
stain fungi 1813
Pulses
aflatoxins 1575
Callosobruchus, India 926
Ephestia cautella, India 926
pest control, ethyl formate 744
Pycnoporus coccineus, timbers, creosotes 1750
Pycnoporus sanguineus
cellulose, biodegradation 631
timbers, resistance 647
Pyralidae, stored products, United Kingdom, books 2080
Pyralis farinalis, flours, biology 1855
Pyrethrins
control, *Lasioderma serricorne* 1934
resistance, *Oryzaephilus surinamensis* 279
wood preservatives 1786
Pyrethroids, resistance, *Sitophilus oryzae* 1861
Pyridines, biodegradation, *Micrococcus luteus* 2011
Pyrocon
sesame
Oryzaephilus surinamensis 974
Tribolium castaneum 974
Pythium pleroticum, ginger, control 1593
Quantitative techniques
aflatoxins, maize 409
liquid chromatography, aflatoxins 411
thin layer chromatography, aflatoxins 410
Quarantine
cucumbers
Dacus cucurbitae 1638
Dacus dorsalis 1638
fruits
Dacus cucurbitae 1597
Dacus dorsalis 1597
pawpaws
Ceratitis capitata 1623
Dacus cucurbitae 1623
Dacus dorsalis 1623
timbers, pest control 212
vegetables
Dacus cucurbitae 1597
Dacus dorsalis 1597
Quarantine certification
fruits, insect pests, USA 497
vegetables, insect pests, USA 497
Quarg, yeasts, detection 1093
Quartz
biodegradation
Bacillus mucilaginosus 814
Thiobacillus thiooxidans 814
Thiobacillus thioparus 814
Quaternary ammonium compounds, sugar products, bacteria 971
Quercus, Ceratocystis fagacearum, methyl bromide 1774
Radioactive waste, bacteria, biodeterioration 2031
Radulum concentricum, timbers 210
Raisins
aflatoxins, drying 1646
Ephestia
Cyprus 1036
insecticides 1036
microbial pesticides 1036
insect pests, controlled atmospheres 1929
Plodia interpunctella
biology 1872
Cyprus 1036
insecticides 1036
microbial pesticides 1036
Rapeseed
Cryptolestes ferrugineus 481
Oryzaephilus mercator 481
Oryzaephilus surinamensis 481
pest control, storage structures 12
Tribolium castaneum 481
Raspberries, *Botrytis cinerea* 524
Rats
control
bromethaline 1909
flocoumafen 1910-1911
Recombination, genes, *Phanerochaete chrysosporium* 376
Reconstituted milk, microbial flora, ultrafiltration 583
Refrigeration
fruits
Dacus cucurbitae 1597
Dacus dorsalis 1597
vegetables
Dacus cucurbitae 1597
Dacus dorsalis 1597
wheat, insect pests 1554
Refuse
composting, Pakistan 326
methane production, reviews 310
Repellents, control, *Sitophilus granarius* 735
Reports
Australian Centre for International Agricultural Research 1495
Centro Internacional de Agricultura Tropical, Cassava program 1390
Danish Pest Infestation Laboratory 2071
foods, safety 381
Hannah Research Institute, United Kingdom 1104
Hawaiian Sugar Planters' Association 1824
Society of Plant Protection of North Japan 1562
stored products pests, United Kingdom 1389
United Kingdom , Tropical Products Institute 822
Resazurin test, milk, microbial count 601
Resins, wood preservatives 1778
Resmethrin, control, *Lasioderma serricorne* 1934
Reticulitermes flavipes
control, bark extracts 1278
timbers, resistance 219
Reticulitermes largus, structural wood, China 1247
Reticulitermes lucifugus, improved wood, resistance 1770
Reticulitermes santonensis
control, cypermethrin 660
improved wood, resistance 1770
Reviews
Aeromonas hydrophila, food poiso 388
Aeromonas sobria, food poisoning
agricultural wastes 2009
algicides, testing 1940
Alternaria, mycotoxins 43-44
Aspergillus clavatus, mycotoxins 1
biogas, production, landfill 1995
cellulases, research 339
cereals
fumigation 1500
insect pests, resistance 1502
wastes, treatment 2037
chemical industries, waste water tr ment 2056
Clostridium botulinum, canned pr ucts 30
coke, industrial wastes 2063
cowpeas, insect pests 1565
cyanides, industrial wastes, treatme 2032
dairy wastes 2054
ethanol, production, lignocellulose
fermentation wastes 2039
fish scrap, treatment 2036
food industry, pest control 1281
foods
amines 854
Campylobacter jejuni 856
coliform bacteria, estimation 40
Salmonella, estimation 405
fruits
postharvest storage decay 126
storage, wraps 104
wastes, treatment 2037
fumigants
modes of action 1290
movements, cereals 754
insecticides, stored products pests 1
lignin, biodegradation 786
marine fouling 694
meatworks effluent, treatment 2036
metals, bacterial leaching 795
milk
post pasteurization contaminatio 1104
quality, Limulus test 573, 575
milk products, *Salmonella* 1684
mycotoxins 1453
estimation 882, 1455
feeds 50
foods 50
thin layer chromatography 55
petroleum, waste water treatment 20
Phialophora, timbers 202
phosphates, meat products, *Clostridi botulinum* 1668
postharvest systems, horticultural cr 495
poultry industry, wastes, treatment 2036
power industry, waste water treatme 2064
psychrotrophic bacteria
enzymes, proteinases 1232
lipase 1837
pulp and paper industry, effluents, treatment 2057
refuse, methane production 310
single cell proteins, production 1997
steel, industrial wastes 2063
storage decay, controlled atmosphere storage 13
stored products, *Prostephanus truncatus,* Africa 1860
stored products pests
biological control 1925
control 755, 1430
pheromones 282
population dynamics 1873
streptomycetes, biodeterioration 699
sugarcane byproducts, utilization 364
surfactants, oils, recovery 354
tea industry, wastes, utilization 805
textiles, waste water treatment 2059
timbers
decay 202
fungal decay 1757
stain fungi, Australia 1391

vs *cont.*
getables
storage 103
wraps 104
wastes, treatment 2037
ıste water treatment
activated sludge 1974
aerobic digestion 1980
anaerobic digestion 1983
biological fixed-film systems 1965
lagoons 1980
ponds 1980
ıste waters
aquaculture 2006
land application 1992
methane production 309
ıter reuse 2006
ood, Agaricales 651
ood destroying fungi, biodeterioration 1756
ood preservation 229
aralenone 1470
octonia
angoes
controlled atmosphere storage 1014
prochloraz 1014
opus
gricultural wastes, utilization 1314
pples, control 505
ontrol, essential oils 1240, 1952
eeds 907
ıaize 901
ıangoes
controlled atmosphere storage 1014
prochloraz 1014
ilk products, Egypt 1094
ycotoxins 54
omegranates, India 1626
roteins, production 1314
pices 490
opus arrhizus, mangoes, control 520
opus artocarpi, *Artocarpus heterophyllus* 1630
opus chinensis, single cell proteins, cassava, poultry manure 331
opus oryzae
ontrol, ammonia 1941
yanides, biodegradation 334
watermelons, India 1640
opus stolonifer
abbages, fungicides 536
oods, analysis 21
ectarines 1011
eaches 1011, 1613
actidione 115
boric acid 115
tone fruits 1616
South Africa 1615
dopseudomonas, citrus, wastes, treatment 1295
dotorula nothofagi, wood 208
zopertha dominica
antifeedants, *Calotropis procera* 1263
cereals
carbon dioxide 1492
damage 57
heating 1504
India 926
insecticides 1511
methyl bromide 1492
control
fumigants 740, 742, 748, 1254
growth regulators 1275
insecticides 1282
methyl chloroform 736
plant extracts 1936
protective atmospheres 1279
2-tridecanone 1865
migration, Australia 714
military rations 855
oats, estimation 1517
pearl millet, varietal susceptibility 945
physiology, aggregation pheromones 1888
resistance
insecticides 719
Australia 1882
malathion 1245, 1901
phosphine 272, 292
pirimiphos-methyl 1901

Rhyzopertha dominica *cont.*
rice
control 66
damage 1512
Lippia geminata 1531
plant extracts 939
susceptibility, amino acids 940
sorghum
biology, development 1545
control 71
insecticides 1567
population dynamics 1499
stored products, Japan 1891
traps 263
wheat
control 75
damage 1512
dichlorvos 1553
ecology, population dynamics 1552
heat treatment 1557
neem extracts 284
plant extracts 1916
population dynamics 1499
refrigeration 1554
uric acid 949
Rice
Corcyra cephalonica
moisture content 455
reproduction 1853
Cryptolestes, control 66
discoloration, control 1535
fungi 942
insect pests
Taiwan 67
traps 1267
microbial flora, temperature 70
mycotoxins, *Fusarium* 413
ochratoxins, estimation 1469
Oryzaephilus surinamensis, biology, reproduction 1533
postharvest losses 465
Rhyzopertha dominica
control 66
damage 1512
plant extracts 939
susceptibility, amino acids 940
Sitophilus oryzae
biology, development 1496
control 66
damage 1512
development 924
plant extracts 939
varietal susceptibility 69, 1532
Sitophilus zeamais
biology, development 1496
oviposition attractants 68
Sitotroga cerealella
dichlorvos 1286
etrimfos 1286
fenitrothion 1286
malathion 1286
plant extracts 939
varietal susceptibility 69, 1534
storage, India 941
stored products pests, *Lippia geminata* 1531
Tribolium castaneum, development 924
Rice straw
biodegradation
Candida acidothermophilum 1956
India 1956
land application 1311, 1988
proteins, production 2001
single cell proteins, production 1956
utilization, *Sporotrichum pulverulentum* 2001
Volvariella diplasia, cultivation, India 815
Rigidoporus lignosus, *Hevea brasiliensis,* lignin, degradation 1758
Road dust, pearl millet, *Ephestia cautella* 943
Rodenticides
brodifacoum, India 274
coumarins 275
Rodents
control
flocoumafen 1911
Kuwait 832
Ronilan Smoke, cabbages, *Alternaria brassicae* 539

Root crops, postharvest losses, Caribbean 500
Rotating biological contactors, waste water treatment 1955
Roundwood
insecticides, spraying 1205
Tomicus piniperda, control 218
wood borers, lime (mineral) 1772
Xyloterus lineatus, slaked lime 221
Rovral, apples, postharvest decay 505
Rubber
biodegradation, *Nocardia* 813
Olpitrichum, enzymes 1214
Torula thermophila, enzymes 1214
wastes, biodegradation, *Pseudomonas acidovorans* 1345
Rumen microorganisms
cellulosic wastes, anaerobic digestion 2014
municipal refuse disposal, anaerobic digestion 2007
Rural sociology, biotechnology 758
Rye
Cryptolestes ferrugineus
malathion 1494
pirimiphos-methyl 1494
S compounds, wood preservatives 668
Sacadodes pyralis, cotton, hydrocyanic acid 1158
Saccharomyces anamensis
enzymes, immobilization 370
whey, utilization 370
Saccharomyces cerevisiae
wheat bran, utilization 806
whey
fermentation 1372, 1377, 2047
utilization 1367
genetic engineering 2053
Saccharomyces diastaticus, potato starch, utilization 1317
Saccharomyces fragilis, whey, fermentation 2049-2050
Saccharomyces lactis, taxonomy 1844
Saccharomyces rouxii, beverages, techniques, detection 491
Saccharomycopsis fibuligera
potato starch
fermentation 1316
utilization 1317
Sacks, insecticides, persistence 291
Salicylic acid, bananas, *Aspergillus niger* 1633
Salmon
Clostridium perfringens, food poisoning 1661
Staphylococcus aureus, canning 1662
Salmonella
beef, cooling 546
caseinates, culture media 1082
chicken meat
culture media 171
decontamination 558
detection
culture media 401
ELISA 403
dried skim milk
culture media 1082
detection 572
feeds
animal products 1475
detection 1434, 1489
food poisoning 1425
foods
culture media 1441
detection 403, 1432, 1434
estimation, reviews 405
immunoassay 869
immunological techniques 396
isolation, culture media 14, 20, 1915
meat
Brazil 1073
ELISA 1664
milk, heat treatment 1718
milk products
German Federal Republic 1684
New Zealand 562
reviews 1684
poultry meat, Egypt 168
rice, temperature 70
sewage sludge, composts 760
survival, cultured milks 176

Salmonella *cont.*
susceptibility, butylated hydroxyanisole 1950
water pollution, rivers 302
Salmonella montevideo, stainless steel, adhesion 237
Salmonella typhimurium
beef
acetic acid 1066
formic acid 1066
cheeses 847
food poisoning 847, 1156, 1425
Italy 1425
chicken meat, polyphosphates 1076
food poisoning, Canada 847
inhibition, lactic acid 176
low fat milk, food poisoning 1710
milk
food poisoning 596-597
formaldehyde 1692
poultry, adhesion 1670
poultry meat, freezing techniques 1077
Salmonella typhosa, milk, hydrogen peroxide 1701
Sampling, automation, poultry meat 172
Sardines, *Staphylococcus aureus,* canning 1662
Satsumas, prestorage treatment, heat 515
Saudi Arabia
barley, fungi 456
maize, fungi 456
meat products, microbial count 550
sorghum, fungi 456
timbers, Isoptera 1765
vegetables, fungi 105
wheat, fungi 456
Sausages
microbial flora 155
quality, storage 1063
Sawdust, *Ganoderma applanatum,* anatomy 635
Scandinavia, wood preservation 1186
Scenedesmus, pig slurry, treatment 330
Schizophyllum commune, growth, water stress 704
School meals, microbial flora, Brazil 11
Schwanniomyces alluvius, potato starch, utilization 1317
Sclerotinia sclerotiorum, *Actinidia deliciosa* 518
Sclerotium rolfsii
enzymes, cellulose digestion 782
fruits, control 1598
guavas 125
Scolytidae
books, pest management 834
distribution, Zambia 264
logs, control 225
timbers 264
Japan, interceptions 656, 1248
Korea Republic, interceptions 215
Sea pollution, industrial wastes, metals 306
Seafoods
Polyplastron multivesiculatum 1058
quality, storage 1059
Seed treatment, soyabeans 475
Septic tank effluent, anaerobic treatment, filtration 1298
Serpula lacrimans
buildings
alkalis 1227
moisture content 1227
control, HCN 239
growth 701
physiology, water relations 1847
Pinus, laboratory tests 1754
timbers, preservation 1812
Serratia
milk products, Egypt 1095
phosphorus, release 804
Sesame
Oryzaephilus surinamensis
attapulgite 974
bromophos 974
etrimfos 974
malathion 974
methacrifos 974
pirimiphos-methyl 974
pyrocon 974
Tribolium castaneum
attapulgite 974
Sesame *cont.*
Tribolium castaneum cont.
bromophos 974
etrimfos 974
malathion 974
methacrifos 974
pirimiphos-methyl 974
pyrocon 974
Sesquiterpene lactones, antifeedants, stored products pests 734
Setomorpha rutella, cereals, China 1509
Sewage
Enterobacteriaceae, USA 761
land application, India 1991
Sewage effluent, water pollution, rivers 302
Sewage sludge
anaerobic treatment 316
aromatic compounds, biodegradation 1355
composting
aeration 1968
design 1970
temperature 763, 1297, 1968
USA 1969
composts, *Salmonella* 760
disposal, oceans 1987
land application 318, 1994
heavy metals, plants 1310
mine spoil, revegetation 1309
plants 1307
USA 1993
stabilization, Oligochaeta 1961
treatment, thermophilic microorganisms 1964
Sewage treatment, activated sludge, bacteria 1299
Sex attractant traps, insect pests, forecasting 265
Shellfish
bacteria 147
Clostridium perfringens, United Kingdom 542
Escherichia coli, United Kingdom 542
food poisoning, algae, toxins 1658
Streptococcus faecalis, United Kingdom 542
Shigella, rice, temperature 70
Shigella flexneri, shrimps, isolation 1659
Shigella sonnei
beef
acetic acid 1066
formic acid 1066
foods, culture media 1443
milk, culture media 1133
Shingles
preservation
alkylammonium compounds 1800
Cu compounds 1800
Cu-Cr-As 1800
Shrimp culture, waste waters, power generation 1384
Shrimps
bacteria, indoles 151
controlled atmosphere storage 152
pastes, aflatoxins, Singapore 37
quality, storage 1059
Shigella flexneri, isolation 1659
Silage
Clostridium 1092
fungi 589
Silica dusts, control, stored products pests 1930
Singapore
groundnuts, aflatoxins 37
shrimps, pastes, aflatoxins 37
Single cell proteins
cellulose, *Aspergillus fumigatus* 340
production
cocoa husks 1999
plant residues 2000
pulp mill effluent 1381
reviews 1997
rice straw 1956
whey 1364, 1376-1377, 2049
Rhizopus chinensis, cassava, poultry manure 331
technology 319
USSR 320
Sinoxylon anale, timbers, control 1766
Siricidae, timbers, control 1179
Sitophilus granarius
cereals
Canada 1491
cold resistance 752
Poland 454
cold resistance 258
control
antifeedants 730-731, 734-735
fumigants 740, 742
methyl chloroform 736
phosphine 1273
repellents 735
2-tridecanone 1865
feeds, German Federal Republic 90
maize 716
monitoring, traps 1890
physiology
digestive system 1895
enzymes 1858
population dynamics 1251
sorghum 716
wheat 716
chlorpyrifos-methyl 921
controlled atmospheres 1556
etrimfos 921
fenitrothion 921
fungi 74
heat treatment 466
malathion 921
methacrifos 921
methyl bromide 1549
pirimiphos-methyl 921
sorptive silica dusts 1558
Turkey 471
ultrasound 733
uric acid 949
varietal susceptibility 469
Sitophilus oryzae
cereals
carbon dioxide 1492
control 285
damage 57
India 926
insecticides 1511
methyl bromide 1492
moisture content 928
plant extracts 1285
Poland 454
control
dill 1288
fumigants 740, 742, 748
growth regulators 739, 1893
insecticides 1282
methyl chloroform 736
plant extracts 1863
protective atmospheres 1279
2-tridecanone 1865
maize
biology, development 1496
plant extracts 1937
vegetable oils 1519
migration, Australia 714
oats, estimation 1517
pearl millet, varietal susceptibility 94
peas
development 473
phytotoxins 1574
physiology
digestive system 1895
enzymes 1858
resistance
carbon dioxide 1266
DDT 1902
pyrethroids 1861
rice
biology, development 1496
control 66
damage 1512
development 924
Lippia geminata 1531
plant extracts 939
varietal susceptibility 69, 1532
sorghum
captan 946
control 71
DDT 946
environmental factors 947
growth regulators 1250
malathion 946

hilus oryzae cont.
rghum *cont.*
neem extracts 922
Pongamia glabra 922
resistance 1543
igna angularis, phytotoxins 1574
heat 73
biological control 1550
biology, development 1496
control 75, 1547
damage 1512
development 924
dichlorvos 1553
fungi 74
heat treatment 466
phosphine 1559
plant extracts 1916
population dynamics 1499
refrigeration 1554
uric acid 949
varietal susceptibility 950-951
hilus zeamais
ereals, cold resistance 752
ontrol
fumigants 746-747
insecticides 737
2-tridecanone 1865
aize
biology, development 1496
control 60
cyfluthrin 935
deltamethrin 289
fenitrothion 935
flucythrinate 61
malathion 935
susceptibility 59
hysiology, digestive system 1895
opulation dynamics 1873
esistance
malathion 1901
pirimiphos-methyl 1901
ce
biology, development 1496
oviposition attractants 68
rghum
susceptibility 59
varietal susceptibility 944
ored products, Japan 1891
heat, biology, development 1496
roga cerealella
ereals
damage 57
India 926
ontrol
fumigants 747
methyl chloroform 736
plant extracts 1869
sterile insect release 1926
aize
flucythrinate 61
varietal susceptibility 937
earl millet, varietal susceptibility 945
ce
dichlorvos 1286
etrimfos 1286
fenitrothion 1286
Lippia geminata 1531
malathion 1286
plant extracts 939
varietal susceptibility 69, 1534
rghum
environmental factors 947
resistance 1543
ored products, Japan 1891
aps 263
heat 73
control 1283
milk, psychrotrophic bacteria, sensory evaluation 1677
ed lime, timbers, *Xyloterus lineatus* 221
ges
naerobic treatment, Methanobacteriaceae 314
nterovirus, isolation 301
nd application 1311, 1988
king, fish, *Vibrio parahaemolyticus* 1660
um acid pyrophosphate, *Clostridium botulinum*, inhibition 27

Sodium benzoate
preservation, beer 1595
preservatives, wines 98
Sodium bicarbonate, fruits, postharvest decay 1598
Sodium bisulphite, wheat, deoxynivalenol 1560
Sodium carbonate, wood preservatives 1199
Sodium chloride
cabbages, *Listeria monocytogenes* 1656
control, aflatoxins 1468
fish, *Clostridium botulinum* 541
Sodium dodecyltriethoxy sulphate, biodegradation 1352-1353
Sodium hypochlorite
pork, psychrotrophic bacteria 158
stainless steel, bacteria 237
Sodium metabisulphite
bananas, *Aspergillus niger* 1633
preservation, beer 1595
preservatives, wines 98
Sodium pentachlorophenoxide
timbers, fungal stains 232
wood preservatives 232
Sodium petroleum sulphonate, biodeterioration 1213
Sodium 2-phenylphenolate
control, *Geotrichum candidum* 522
lemons, *Geotrichum candidum* 522
oranges, *Penicillium digitatum* 1023-1024
pears, storage decay 506
Soft cheese, microbial flora, Iraq 1676
Soft drinks, *Acetobacter*, flocculation 99
Soil properties, municipal refuse disposal, land application 769
Soils, wood, biodeterioration 646
Solenopsis xyloni, diazinon, almonds 975
Solid waste
composting
Bacillus 778
Pakistan 326
thermophilic bacteria 777
methane production 310
Solvents, production, whey 1374-1375
Sorbic acid
control
fungi 1920
Geotrichum candidum 522
stored products pests 1920
yeasts 25
Cottage cheese, microbial flora 1157
spore germination, inhibition, *Clostridium botulinum* 406
wines
bacteria 991
yeasts 991
Sordaria alcina, straw, biodegradation 342
Sordaria fimicola, beech 652
Sorghum
arthropod pests 1542
Brazil 1541
Corcyra cephalonica
ecology 1544
moisture content 455
reproduction 1853
Cryptolestes ferrugineus, insecticides 1567
Ephestia cautella, control 71
fungi 948
Egypt 72
Saudi Arabia 456
insect pests
Africa 1537
Australia 1540
India 1538
South East Asia 1539
mycotoxins, *Fusarium* 413
Oryzaephilus surinamensis, insecticides 1567
Rhyzopertha dominica
biology, development 1545
control 71
insecticides 1567
population dynamics 1499
Sitophilus granarius 716
Sitophilus oryzae
captan 946
control 71
DDT 946
environmental factors 947

Sorghum *cont.*
Sitophilus oryzae cont.
growth regulators 1250
malathion 946
neem extracts 922
Pongamia glabra 922
resistance 1543
Sitophilus zeamais
gamma radiation 59
susceptibility 59
varietal susceptibility 944
Sitotroga cerealella
environmental factors 947
resistance 1543
Tribolium castaneum
control 71
ecology 1544
insecticides 1567
Trogoderma granarium, insecticides 1567
Sorptive coatings, control, stored products pests 1930
Sorptive silica dusts, wheat, insect pests 1558
Sour milk
microbial flora, Iraq 169
Salmonella, heat treatment 1718
South Africa
cereals, mycotoxins 875
feeds, mycotoxins 875
interceptions
Heterobostrychus aequalis, timbers 211
Xylothrips flavipes, timbers 211
mycotoxins, legislation 35
stone fruits
Botrytis cinerea 1615
Monilinia laxa 1615
Rhizopus stolonifer 1615
wood preservation 1187
South East Asia
cereals, storage 1495
insecticides, degradation 751
sorghum, insect pests 1539
Soyabean oilmeal, zearalenone, sampling 441
Soyabeans
aflatoxins 36, 41, 1581
bibliographies 86
Callosobruchus maculatus, biology, development 1572
fungi 87
captan 475
heating 1579
insect pests
Israel 1506
monitoring 1506
prediction 1562
Spain
feeds, aflatoxins 1467
foods, aflatoxins 1467
milk, *Listeria* 1131
Spectrophotometry, dichlorophen 231
Sphaerotilus, cooling systems, fouling 1230
Spices
aflatoxins 95
fungi 490
insect pests, India 853
microbial count
determination 865
Pakistan 988
microflora 489
pest control, ethyl formate 744
quality, USA 989
Spirulina maxima
alcoholic beverages, wastes, fermentation 808
distillery effluents, utilization 2038
Spoilage
indexes
Coryphaena hippurus 154
fish 154
Spore germination, inhibition, *Clostridium botulinum* 406
Sporeforming bacteria, milk, cheeses, sensory evaluation 1715
Spores
bacteria, heat resistance, determination 726
germination, *Clostridium perfringens* 248

Sporotrichum pulverulentum
bagasse, biodegradation 811
rice straw, utilization 2001
timbers, wood anatomy 636
Sri Lanka, foods, bacterial count 848
Stachybotrys atra, beech 652
Stain fungi
canes and rattans
drying, equipment 1166
PCP-Na 1166
pulping materials 1813
timbers
Australia, reviews 1391
benomyl 1171
control 667
Fiji 667
PCP 1171
preservation 1206
pulping 1813
thiophanate-methyl 1171
wood preservation 205
wood chips, pulping 1813
Stainless steel
adhesion
Bacillus cereus 237
Pseudomonas fragi 237
Salmonella montevideo 237
bacteria, sodium hypochlorite 237
Standards
ceramic tile adhesives, microbial contamination 387
milk, microbial flora 174
paper, biodeterioration, USSR 1211
poles, preservation, Taiwan 1805
wood preservation
German Federal Republic 670
New Zealand 1190
terminologies 1188
USSR 1781
wood preservatives
German Federal Republic 1204
USA 1411-1422
Staphylococcus
beef, Nigeria 161
cheeses 1153
identification 1726
enterotoxins
detection, immunoassays 1449
foods, Netherlands 1446
meat 1446
infant formulae, Nigeria 1673
milk 1123
culture media 1107
heat treatment 1097
milk products, India 1091
Staphylococcus aureus
dried milk
detection 184
enumeration, culture media 594
enterotoxins
cheeses 1727
foods, detection, thermonuclease 1448
ice cream 871
Argentina 1679-1680
food poisoning, Iran 1426
food products, Nigeria 10
foods
antibiotics, resistance 246
culture media 1435, 1445
isolation, meat 1071
milk 610
formaldehyde 1692
milk products
detection 397
enterotoxins 175
poultry meat, Egypt 168
rice, temperature 70
salmon, canning 1662
sardines, canning 1662
susceptibility, butylated hydroxyanisole 1950
textiles, adhesion 1817
Staphylococcus epidermidis, textiles, adhesion 1817
Steel
antifouling agents
bacteria 1228
bacterial leaching 1229
bacteria
seawater 1223
Steel *cont.*
bacteria *cont.*
USSR 1225
Desulfovibrio desulfuricans 1221
soil 1224
industrial wastes, reviews 2063
Thiobacillus thioparus, soil 1224
Stegobium paniceum
lentils, development 1563
stored products, Australia, New South Wales 1868
Stereum, plywood, laboratory tests 639
Stereum frustulosum, cellulose, biodegradation 631
Stereum hirsutum
Fagus 648, 650
timbers, logging, seasonal variation 640
Stolotermes inopinus, timbers, New Zealand 1763
Stolotermes ruficeps, timbers, New Zealand 1763
Stone fruits
Botrytis cinerea 1616
South Africa 1615
Monilinia laxa 1616
South Africa 1615
Rhizopus stolonifer 1616
South Africa 1615
Stones, weathering, lichens, organic acids 256
Stonework, biodeterioration, Italy 1823
Storage
diseases
apples, France 1003
pears, France 1003
quality, peaches, calcium 512
wraps
fruits, reviews 104
vegetables, reviews 104
Storage decay
controlled atmosphere storage, reviews 13
cowpeas 1578
groundnuts, plant extracts 1949
onions 1040
potatoes, controlled atmosphere storage 1046
soyabeans 475
vegetables, packhouses 499
Storage dips
Alternaria 1031
citrus fruits, decay 1621
Fusarium 1031
melons 1031
Storage losses, maize, *Prostephanus truncatus,* Tanzania 63
Stored products
Anthrenus verbasci, Australia, New South Wales 1866
arthropod pests, Indonesia 1246
Attagenus unicolor, Australia, New South Wales 1866
Coleoptera, India 1252
Gibbium aequinoctiale, distribution 1257
Gibbium psylloides, distribution 1257
insect pests
Japan 1891
monitoring 263, 276
insects, Indonesia 1246
Lasioderma serricorne, Australia, New South Wales 1868
mites
biological control 1905
China 1907
Egypt 1908
taxonomy 1906
Oryzaephilus surinamensis, United Kingdom 1890
pest control
permethrin 63
timing 296
Plodia interpunctella, Australia, New South Wales 1871
Prostephanus truncatus, Africa, reviews 1860
Pyralidae, United Kingdom, books 2080
research, United Kingdom, reports 822
Stegobium paniceum, Australia, New South Wales 1868
Stored products *cont.*
Tribolium castaneum, United Kin 1890
Tyrophagus longior, Poland 724
Stored products pests
books 838
Canada 1852
cereals
aeration 925
monitoring, traps 1437
conferences 2076
control 1931
abamectin 1885
bibliographies 1951
biological control 1927
reviews 1925
carbon dioxide 1928
controlled atmospheres 1928
fenoxycarb 1919
fumigation, USSR 295
insecticides 1917
reviews 1918
pheromones, reviews 282
phosphine 1924
reviews 755, 1430
sorbic acid 1920
sterile insect release 1926
techniques 1501
Denmark 2071
detection 1436
grain stores, historical records 151
monitoring, traps 1874, 1890
population dynamics
reviews 1873
techniques 1876
resistance
fumigants 1922
malathion, Canada 1921
spread 6
traps
light traps 1261
sex attractant traps 296
United Kingdom, reports 1389
Straw
biodegradation 785, 1344
Cellulomonas 1339-1340
Cellulomonas gelida 1340
fungi 342
feeds, production 1318
fermentation, *Lyophyllum decaste* 1318
fungi 1485
recycling, *Pleurotus* 1341
upgrading
Chaetomium cellulolyticum 13
Penicillium verruculosum 1319
utilization, nitrogen fixation, *Azosp lum brasilense* 1339-1340
Straw treatment, *Aspergillus glaucus*
Strawberries, *Botrytis cinerea,* process wines 1636
Streptococcus
cheeses 623
culture media 618
condensed milk, heat resistance 56
dried skim milk, heat resistance 56
ewe milk, Bulgaria 1697
infant formulae, heat resistance 56
milk 1123
Streptococcus faecalis
beef
acetic acid 1066
formic acid 1066
poultry meat, Egypt 168
shellfish, United Kingdom 542
Streptococcus lactis
meat products, microbial count 10
milk
formaldehyde 1692
hydrogen peroxide 1701
Streptococcus thermophilus, milk, for dehyde 1692
Streptomyces
biodeterioration, reviews 699
waste water treatment, food indust 1302
Streptomyces viridosporus, lignocellul biodegradation 1733
Streptomycetes, biodeterioration, revie 699

:tural timbers
nobium punctatum
German Democratic Republic 1769
ionizing radiation 1769
reservation
China 1833
tributyltin oxide 1948
:tural wood
ryptotermes domesticus, India 654
ryptotermes dudleyi, India 654
migation, HCN 239
terotermes occidentis 216
teliculitermes largus, China 1247
r factory waste
zotobacter, waste water treatment 308
onversion
Arthrobacter globiformis 812
17-ketosteroids 812
nd application 1311
r products, bacteria, quaternary ammonium compounds 971
rbeet seeds, *Tyrophagus putrescentiae* 712
rcane, mycotoxins, *Fusarium* 413
rcane byproducts, utilization, reviews 364
rcane pith, digestion, feeds 322
rs, production, cellulosic wastes 2018
asia pontifica, cashews, New Zealand, interceptions 1900
obacillus thermosulfidooxidans, heating systems, USSR 1225
henamides, wood preservatives 668
hides
eaching, *Thiobacillus ferrooxidans* 1382
xidation
Aspergillus niger 1346
Trichoderma harzianum 1346
hur dioxide
ontrol, yeasts 25
rapes, storage 1029
huryl fluoride, control, insect pests 1290
bark E, logs 225
lower oil, control, *Callosobruchus maculatus* 81
lower seeds, fungi 1584
actants
Bacillus licheniformis, oils, recovery 356
iodegradation 1352-1353
ils, recovery, reviews 354
ziland, wood preservation 1187
den
ooks, fungi 1832
omes, fungi 240
oles, decay 1163
et potatoes
Botryodiplodia theobromae
enzymes 531
physiology 532
zerland, wood preservation, methodology 1794
id 244, control, stored products pests 1930
metrischema plaesiosema, potatoes, control 1042
hetic fibres, bacteria, adhesion 1817
S-T, sewage sludge, biodegradation 1355
van
ereals, mycotoxins 1461
eeds, mycotoxins 1461
oods, mycotoxins 1461
oles, preservation, standards 1805
ice, insect pests 67
romyces flavus, fruit products, Czechoslovakia 997
gors
Penicillium digitatum
fungicides 1621
packaging 1621
waxes 1621
torage decay, control 1025
nery waste, land application, plants 1383
nins, biodegradation, bacteria 332
zania
uildings, wood preservation 1737

Tanzania *cont.*
cassava, *Prostephanus truncatus* 530, 1892
maize
Prostephanus truncatus 1892
storage losses 63
timbers
durability 1738
Isoptera 1738
Tars
wood preservatives 661, 1412, 1420
piles 1801
TCMTB, wood preservatives 637, 1804
TCP
timbers, fungi 1810
wood preservatives 205, 1810
Tea industry, wastes, utilization, reviews 805
Techniques
beverages, yeasts, detection 491
fumigation 677
microbial flora, foods 16
mites, feeds, determination 400
pork, bacterial count 159
reconstitution, dried foods, microbial count 402
stored products pests, population dynamics 1876
wood, decay 1913-1914
Technology, single cell proteins 319
Tempeh, microbial contamination, storage 1594
Tenebrio, distribution, Caribbean 267
Tenebrio molitor
cereals
Canada 1491
Poland 454
physiology, enzymes 1858
wheat, heat treatment 466
Tenebroides mauritanicus, identification 259
Tensile strenth, testing, textiles 680
Terminalia, Penicillium 122
Tetrachloroethylene, biodegradation, bacteria 1328
Tetrachlorvinphos
control, *Lasioderma serricorne* 291
dried fish
Dermestes maculatus 1056
Necrobia rufipes 1056
Textiles
bacteria, adhesion 1817
tensile strenth, testing 680
Tinea pellionella, Australia, New South Wales 1815
Tineola bisselliella, Australia, New South Wales 1815
waste water treatment, reviews 2059
Thailand
cereals, storage 1495
hemp, insects 1728
Thamnidium elegans, beef, antibacterial properties 252
Thatch
fungicides, copper sulphate 690
Phragmites australis, biodeterioration 242
wood preservation 666
Thermoascus aurantiacus, cellulose, biodegradation 343
Thermoduric bacteria
milk 593
cheeses, sensory evaluation 1715
Egypt 1127
Thermomonospora curvata, D-xylan, biodegradation 2013
Thermomonospora fusca, D-xylan, biodegradation 2013
Thermomyces lanuginosus, cellulose, biodegradation 343
Thermophilic actinomycetes, wood chips, storage 1170
Thermophilic bacteria
solid waste, composting 777-778
whey, utilization 2042
Thermophilic fungi, taxonomy 702
Thermophilic microorganisms, sewage sludge, treatment 1964
Thermoproteus, heating systems, USSR 1225

Thiabendazole
apples, postharvest decay 505
citrus fruits, *Penicillium digitatum* 120
nectarines, postharvest decay 1011
oranges
Penicillium 514
Penicillium digitatum 1023-1024
peaches, postharvest decay 1011
pears, storage, diseases 1605
potatoes
bacteria 1043
Fusarium 1043
residues 514
timbers, fungi 1810
wood preservatives 1810
Thielavia
beet pulp, utilization 1315
cellulolytic microorganisms 1237
enzymes, cellulases 1237
proteins, production 1315
Thin layer chromatography
aflatoxins, determination 877
mycotoxins, reviews 55
Thiobacillus ferrooxidans
arsenic, leaching 1324
sulphides, leaching 1382
Thiobacillus thiooxidans, quartz, biodegradation 814
Thiobacillus thioparus
quartz, biodegradation 814
steel, soil 1224
Thiometon, wood preservatives 659
Thiophanate-methyl, timbers, stain fungi 1171
Thiosphaera pantotropha, waste water treatment 768, 803
Tilachlidium medietatis, apples, controlled atmosphere storage 1007
Tilapia, microbial count, ice 150
Timbers
Anobium punctatum
German Democratic Republic 1769
ionizing radiation 1769
Arthrographis cuboidea 232
bacteria 1740
basidiomycetes
German Democratic Republic 1743
taxonomy 1743
Bostrichidae, Korea Republic, interceptions 214
Camponotus abdominalis, USA, Florida 1265
Cerambycidae, India 1249
Ceratocystis fagacearum, methyl bromide 1774
Cerrena unicolor, wood chemistry 200
Chaetomium globosum, resistance 645
Coleoptera 1182
Coniophora puteana
boron 678
F compounds 1790
petroleum oils 1200
resistance 645
wood properties 203
control 225
Coptotermes acinaciformis
Australia 1764
New Zealand 1764
Coptotermes formosanus
consumption 1767
resistance 657
Coptotermes frenchi
Australia 1764
New Zealand 1764
Coriolus, wood chemistry 1172
Coriolus versicolor 1209
durability 1168
hydrogen peroxide 1175
physiology 1752
preservation 1812
resistance 645, 1744
wood chemistry 1747
wood properties 203
Cryptotermes cynocephalus, resistance 1771
Cytospora pini, wood cement 638
decay
Cu-Cr-As 1210
detection 689
field tests, USA 633
preservation 637

Timbers *cont.*
decay *cont.*
resistance, Paraguay 1744
reviews 202
water storage 632
wood moisture 1167
decay fungi
books 840
logging, seasonal variation 640
resistance 647
strength 644
durability, Tanzania 1738
fumigation, HCN 239
fungal decay, reviews 1757
fungi 1161
Bulgaria 1741
control 199
detection, spectrometry 1745
methyl bromide 224
oxygen requirement 1762
preservation 1810
USA 643
wood preservation 205
Ganoderma applanatum
chloropicrin 223
wood chemistry 200, 1169
Ganoderma lucidum, decay 1760
Ganoderma tsugae, decay 1760
Gloeophyllum saepiarium 633
physiology 1752
Gloeophyllum trabeum
detection 1165
durability 1168
physiology 1752
preservation 1793
resistance 1744
Heterobostrychus aequalis, South Africa, interceptions 211
Hylotrupes bajulus, phosphine 688
Hyphoderma mutatum 1209
Inonotus rheades, wood chemistry 1172
insect pests
Australia, books 1402
Austria, books 1403
books 840
control 199, 1766
Korea Republic, interceptions 215
insecticides, spraying 1205
insects 1161
control 1768
Irpex lacteus 633
India 1742
Ischnoderma resinosum, wood chemistry 200
Isoptera
camphor 1242
dichlorvos 1242
Guam 1773
India, Gujarat 1903
insecticides 1765
malathion 1242
Saudi Arabia 1765
Tanzania 1738
wood preservation 658, 1773
Kalotermes brouni, New Zealand 213
Kalotermes flavicollis, resistance 1770
Lecythophora mutabilis, Cu-Cr-As 1759
Lentinula edodes 1174
Lentinus lepideus
creosotes 1750
physiology 1752
marine borers, preservation 220
marine fungi, USSR 1177
Meruliopsis corium 633
Microcerotermes bresoni, acetylation 1780
Microtermes, susceptibility 217
Odontotermes, susceptibility 217
Peniophora
wood anatomy 642
wood chemistry 642
Peniophora cinerea 633
Peniophora sanguinea, wood chemistry 1172
Peniophora violaceo-livida 633
Perenniporia tephropora, creosotes 1750
pest control, quarantine 212
Phallus impudicus 206
Phanerochaete chrysosporium 633
Timbers *cont.*
Phanerochaete sanguinea, wood chemistry 1172
Phellinus igniarius, strength of wood 204
Phellinus noxius, lignin, degradation 1758
Phialophora, reviews 202
Phialophora japonica 255
Phlebia brevispora, physiology 1752
Phlebia tremellosus, decay 1761
Phlebiopsis gigantea 1209
Platypodidae, Japan, interceptions 656, 1248
Platypus, New Zealand 1775
Polyporus palustris, laboratory tests 1748
Poria, physiology 1752
Poria carbonica
chloropicrin 223
methyl isothiocyanate 677
physiology 1752
Poria medulla-panis, wood chemistry 200
Poria monticola
acetylation 1780
wood properties 203
Poria placenta
hydrogen peroxide 1175
physiology 1752
resistance 1744
Poria radiculosa, physiology 1752
Poria xantha, physiology 1752
preservation 673, 1801, 1809
alkylammonium compounds 1800
As compounds 1807
Cr compounds 1807
Cu compounds 1800
Cu-Cr-As 1791, 1800
durability 1791
fumigants 1796
pressure treatments 671-672
United Kingdom 1787
Zn compounds 1807
Pterotermes occidentis 216
Pycnoporus coccineus, creosotes 1750
Radulum concentricum 210
resistance 1047
Reticulitermes flavipes, resistance 219
Reticulitermes lucifugus, resistance 1770
Reticulitermes santonensis, resistance 1770
Rigidoporus lignosus, lignin, degradation 1758
Scolytidae 264
Japan, interceptions 656, 1248
Serpula lacrimans
laboratory tests 1754
preservation 1812
Sporotrichum pulverulentum, wood anatomy 636
stain fungi
Australia, reviews 1391
benomyl 1171
control 667
Fiji 667
PCP 1171
preservation 1206
pulping 1813
thiophanate-methyl 1171
Stolotermes inopinus, New Zealand 1763
Stolotermes ruficeps, New Zealand 1763
Tomicus piniperda, control 218
Trametes lilacino-gilva 1753
wood anatomy 1751
Trametes trogii, India 1742
Trichoderma 1174
Tyromyces palustris, preservation 1812
wood borers
Canada 1180-1181
control 199, 1179
lime (mineral) 1772
Poland 659
wood preservation 222, 662, 1811
methodology 226
Xylaria 633
Xyloterus lineatus, slaked lime 221
Timbers *cont.*
Xylothrips flavipes, South Africa, interceptions 211
Tinea pellionella, textiles, Australia, New South Wales 1815
Tineidae, yams, Ivory Coast 1647
Tineola bisselliella
physiology, enzymes 1858
textiles, Australia, New South Wa. 1815
wool textiles
fenvalerate 679
permethrin 1816
Titanocenes, control, stored products p. 1884
Tobacco
Ephestia elutella, dichlorvos 985
Lasioderma serricorne
cooling 1591
methoprene 1590
storage decay 986
Togo, maize, *Prostephanus truncatus*
Toluene, biodegradation, anaerobic digestion 2020
Tomato concentrate, analytical method microorganisms, enumeration 130
Tomato products, fungi, estimation 164
Tomatoes
alternariol methyl ether, estimation 1642
Aspergillus flavus, enzymes 131
Aspergillus fumigatus, enzymes 13
Botrytis cinerea, ethylene 1641
Cladosporium oxysporum, control 1644
Penicillium, ethylene 1641
postharvest decay, USA 1034
postharvest treatment, fungicides 1
prestorage treatment, iodine 510
storage
controlled atmosphere storage 1
plastic film 994
storage decay, plastic film 527
tenuazonic acid, estimation 1642
Tomicus piniperda
timbers
control 218
lime (mineral) 1772
Torula thermophila, rubber, enzymes 1
Torulopsis candida, whey, utilization 1
Toxic substances
activated sludge, bacteria 312
biodegradation 1963
Toxinogenic fungi
barley 461
cereals 883
coffee, Japan 486
control, diacetyl 1452
ecology 419
feed grains, India 1480
foods 48
bioassays 443
China 7
maize, Iraq 1522
malt 461
pecans 977
wheat straw 883
Toxins
algae, shellfish 1658
cheese spread, *Clostridium botulinu* 29
Clostridium botulinum
bacon 157
beef 32
detection 31
immunoassay 407
pH 873
water content 873
microorganisms, foods, conferences 2075
Trametes lilacino-gilva
Eucalyptus 1753
wood anatomy 1751
Trametes trogii, timbers, India 1742
Traps
cereals, insect pests 714
sex attractant traps, stored products pests 296
Trentepohlia odorata
control, biocides 695
paints, control 695

Azines, *Pseudomonas aeruginosa,* resistance 297
Azophos, timbers, insect pests 658
Tribolium
control, insecticides 1243
distribution, Caribbean 267
flour mills, Cyprus 1243
resistance
malathion 753
phosphine 272
stored products, Japan 1891
Tribolium castaneum
aggregation pheromones 709
antiaggregation pheromones 718
cereals
Canada 58, 1491
carbon dioxide 1492
India 926
insecticides 1511
methyl bromide 1492
cold resistance 258
control
biological control 727
chlordimeform 290
DEF 290
fumigants 728, 740, 742, 746-748
gamma radiation 713, 727-728, 1255
growth regulators 1275
insecticides 737, 1282
malathion 713, 1935
permethrin 290
plant extracts 1898
protective atmospheres 1279
sorptive coatings 1930
titanocenes 1884
2-tridecanone 1865
triphenyl phosphate 1935
ultraviolet radiation 1856
lentils, development 1563
migration, Australia 714
military rations 855
mills, dichlorvos 1272
monitoring, traps 1890
oats, methoprene 1516
physiology
enzymes 1858
integuments 1880
population dynamics 1873
raisins, controlled atmospheres 1929
rapeseed 481
resistance
insecticides 719
Australia 1882
malathion 717, 1245
Canada 1921
phosphine 1255, 1933
rice, development 924
sesame
attapulgite 974
bromophos 974
etrimfos 974
malathion 974
methacrifos 974
pirimiphos-methyl 974
pyrocon 974
sorghum
control 71
ecology 1544
insecticides 1567
sterilization, plant extracts 1883
stored products, United Kingdom 1890
traps 263
aggregation pheromones 715
wheat
chlorpyrifos-methyl 921
control 75
cypermethrin 1548
development 924
dichlorvos 1553
embelin 470
etrimfos 921
fenitrothion 921
fenvalerate 1548
malathion 921
methacrifos 921
pirimiphos-methyl 921
refrigeration 1554
sorptive silica dusts 1558
varietal susceptibility 954
wheat flour, growth regulators 1582
Tribolium confusum
cereals, Poland 454
control
antifeedants 730-731, 734
dill 1288
fumigants 742
gamma radiation 1938
growth regulators 1275
insecticides 737
methyl chloroform 736
plant extracts 1863
sorptive coatings 1930
2-tridecanone 1865
resistance, insecticides, Australia 1882
traps 276
wheat
control 1547
proteins 467
Turkey 471
Tribromophenol, wood preservatives 1812
Tributyltin oxide
detoxification, *Phialophora* 1948
wood preservatives 230, 662, 1948
Tricalcium phosphate
cowpeas, *Callosobruchus maculatus* 1566
Phaseolus vulgaris
Acanthoscelides obtectus 1566
Callosobruchus chinensis 1566
Callosobruchus maculatus 1566
Trichlorfon
control
Ceratitis capitata 1253
Lasioderma serricorne 1934
Trichloroethylene
biodegradation
Acinetobacter 2012
bacteria 1328
2,4,6-Trichlorophenol, sewage sludge, biodegradation 1355
Trichlorphon, timbers, wood borers 1179
Trichoderma
control, polyoxin 1843
polyvinyl chloride 1217
timbers 1174
Trichoderma album, cellulosic wastes, fermentation 2017
Trichoderma harzianum
2,3-butanediol, production 790
cellulose, biodegradation 790
metals, extraction 1346
straw, biodegradation 342
sulphides, oxidation 1346
Trichoderma lignorum
agricultural wastes, anaerobic treatment 329
cellulolytic microorganisms 1237
enzymes, cellulases 1237
Trichoderma longibrachiatum, enzymes, cellulolytic enzymes 1846
Trichoderma reesei
cellulose, models 1335
cellulose digestion, enzyme activity 349
mutants, cellulases 819
Trichoderma viride
cellulolytic microorganisms, culture media 816
cellulosic wastes, utilization 2018
taxonomy 1844
Tricholomopsis platyphylla, Fagus 649
Trichosporon beigelii, organic acid recovery system, biofouling 1835
Trichosporon cutaneum, whey, fermentation 1376
Trichothecium, feeds, USSR 911
Trichothecium roseum
fruits, physiology 106
pawpaws, biochemistry 1634
2-Tridecanone, control, stored products pests 1865
Tridomyrmex anceps, pallets, New Zealand, interceptions 1899
Triforine
apples, postharvest decay 505
bananas, *Colletotrichum musae* 1026
Trimethylamine oxide, reduction, prawns, bacteria 540
Triphenyl phosphate, control, *Tribolium castaneum* 1935
Triticale
Cryptolestes ferrugineus
malathion 1494
pirimiphos-methyl 1494
Trogoderma granarium
biology 722
cereals, plant extracts 1285
control
antifeedants 730-731, 734
fumigants 271, 742
protective atmospheres 1279
titanocenes 1884
Coriandrum sativum 94
development
humidity 711
temperature 711
feeds, German Federal Republic 908
lentils, development 1563
pearl millet, etrimfos 1536
resistance, phosphine 1886
sorghum, insecticides 1567
wheat
heat treatment 466
proteins 467
varietal susceptibility 952
Trogoderma variabile
cereals, Australia, New South Wales 1867
control, phosphine 1262
military rations 855
traps 276
Tropics
crops, losses, pest control 1
wood preservation, books 1401
Trypodendron lineatum, logs, Canada 1180-1181
Tsuga heterophylla, Ganoderma applanatum, anatomy 635
Turkey
feeds, aflatoxins 915
wheat, insect pests 471
Turmeric, insecticidal properties 1244
Typha latifolia, waste water treatment 378
Tyromyces palustris, timbers, preservation 1812
Tyrophagus longior, stored products, Poland 724
Tyrophagus putrescentiae, sugarbeet seeds 712
UHT milk, microbial contamination, detection 185
UHT treatment
milk
Bacillus 586
quality 1100
Ultrasound
control, stored products pests 732-733
wheat
insect pests 732
Sitophilus granarius 733
Ultraviolet radiation
control, *Tribolium castaneum* 1856
milk, microbial contamination 1705
packaging, decontamination 685
United Kingdom
apple juice, patulin 993
books, field crops, pests 836
buildings
biodeterioration, books 383
preservation 1787
cereals
deoxynivalenol 434
insect pests 453
mycotoxins 1514
pest control 921
culture collection catalogues, National Collection of Industrial Bacteria 1408
feeds
mites 909
mycotoxins 451
food industry, Psocoptera 1258
foods
food hygiene, inspection 395
Psocoptera 1259-1260
goat milk, microbial flora 186
grain stores, insects 920
industrial wastes, tipping 327
landfill, methane production 770
meat, ochratoxins 1064
milk, aflatoxins 1147

United Kingdom *cont.*
murals, *Beauveria alba* 1829
nut products, aflatoxins 972
organotin compounds, paints, biofouling 692
peaches, *Euzophora bigella* 1614
Pyralidae, stored products, books 2080
shellfish
Clostridium perfringens 542
Escherichia coli 542
Streptococcus faecalis 542
stored products
Oryzaephilus surinamensis 1890
Tribolium castaneum 1890
stored products pests, reports 1389
warehouses, *Ephestia* 1889
Uranium
bacterial leaching, cyanobacteria 796
mine spoil, bacteria, biodeterioration 2031
Urocanic acid
production
Coryphaena hippurus 154
fish 154
USA
bridges, decay 689
canning industry, *Clostridium botulinum* 247
cereals, storage 1508
culinary herbs, quality 989
culture collection catalogues 1410
feeds, deoxynivalenol 1464
foods
deoxynivalenol 1464
mycotoxins, legislation 2
fruits, insect pests, quarantine certification 497
groundnuts, insect pests 1587
irrigation equipment, Formicidae 1824
meat
Campylobacter coli 160
Campylobacter jejuni 160
melons, postharvest decay 1639
methane production, technology 323
milk
bacterial count 1128
post pasteurization contamination, health hazards 1716
municipal wastes, composting 1967
poles, fungi 643
sewage, Enterobacteriaceae 761
sewage sludge
composting 1969
land application 1993
sludges, enterovirus 301
spices, quality 989
standards, wood preservatives 1411-1422
timbers, decay, field tests 633
tomatoes, postharvest decay 1034
vegetables, insect pests, quarantine certification 497
wheat, deoxynivalenol 955
wood preservation, quality controls 1192
USSR
agricultural buildings
biodeterioration 839
books 839
Anobiidae, books 1400
cereals, mycotoxins 1461
feeds
fungi 911
mycotoxins 1461
foods
aflatoxins 438
mycotoxins 1461
fruits, fungi 998
heating systems, bacteria 1225
lakes, cellulolytic microorganisms 787
single cell proteins 320
standards, paper, biodeterioration 1211
stored products pests, control, fumigation 295
timbers, marine fungi 1177
vegetables, fungi 998
wood preservation, standards 1781
Vacuum packaging, potatoes, *Clostridium botulinum* 534
Vacuums
cereals, insect pests 1505
Vacuums *cont.*
control, *Tribolium confusum* 1938
Veal
bacteria, control 1072
decontamination, lactic acid 551-552
Vegetable oils
maize, *Sitophilus oryzae* 1519
Vigna radiata, Callosobruchus chinensis 1573
Vegetables
Dacus cucurbitae
quarantine 1597
refrigeration 1597
Dacus dorsalis
quarantine 1597
refrigeration 1597
fungi
India 1599
Saudi Arabia 105
USSR 998
insect pests
irradiation 496
quarantine certification, USA 497
microbial contamination, controlled atmosphere storage 107
microbial count, Egypt 538
patulin 49
pest control, storage structures 12
postharvest decay, India 498
postharvest losses, Caribbean 500
postharvest systems, reviews 495
storage 380
reviews 103
wraps, reviews 104
storage decay, packhouses 499
wastes, treatment, reviews 2037
Yersinia enterocolitica, virulence 697
Veneers, decay, laboratory tests 1164
Venezuela, feeds, aflatoxins 913-914
Venturia inaequalis, apples, control 1004, 1603
Verticillium, polyvinyl chloride 1217
Vibrio
Callinectes sapidus 153
organotin compounds, leaching 1229
Vibrio parahaemolyticus, fish, smoking 1660
Vicia faba
Bruchus pisorum, New Zealand, interceptions 1899
Callosobruchus chinensis, resistance 1568
Vigna aconitifolia, fungi 84
Vigna angularis
Callosobruchus chinensis 78
gamma radiation 474
resistance 1568
Callosobruchus maculatus 78
Sitophilus oryzae, phytotoxins 1574
Vigna mungo, Callosobruchus, India 964, 966-967
Vigna radiata
Callosobruchus, India 964, 966-967
Callosobruchus chinensis 965
biology 962
deltamethrin 1569
piperonyl butoxide 1569
resistance 1568
vegetable oils 1573
Callosobruchus maculatus 965
biology 962
development 1570
fungi 85
Vigna unguiculata, Callosobruchus maculatus, population density 968
Vinclozolin, pears, storage, diseases 1605
Vinegar, *Acetobacter aceti* 990
Vinyl chloride, biodegradation, bacteria 1328
Vinylidene chloride, biodegradation, bacteria 1328
Vitamin B12
production
citrus, wastes 1360
molasses 2033
whey 2033
Volvariella diplasia, cultivation, rice straw, India 815
Waferboard, preservation, testing 1195
Walls
paints
Cladosporium 1821
Phialophora verrucosa 1821
Warehouses, *Ephestia,* United Kingdom 1889
Waste treatment
bacteria 1295
toxic substances, microbial degradation 1963
Waste utilization, feeds 329
Waste water treatment
activated sludge
computer program 1979
reviews 1974
aerobic digestion, reviews 1980
anaerobic digesters 1986
anaerobic digestion 1984
reviews 1983
Azotobacter
pulp mill effluent 308
sugar factory waste 308
bacteria 1294
biofilms 307
biological filtration, *Eichhornia crassipes* 1971-1972
biological fixed-film systems, reviews 1965
chemical industries, reviews 2056
Egeria densa 378
Eichhornia crassipes 378-379
food industry
Bacillus 1302
Oerskovia 1302
Streptomyces 1302
health hazards 1957
Hydrocotyle umbellata 378
kinetics 1960
Klebsiella 768
lagoons
plants 1304
reviews 1980
peat, pressing 366
petroleum, reviews 2055
phenols, ligninase 371
phosphorus
removal
Acinetobacter 2030
activated sludge 2030
ponds, reviews 1980
power industry, reviews 2064
rotating biological contactors 1955
sequencing batch reactors 1953-1954
textiles, reviews 2059
Thiosphaera pantotropha 768, 803
Typha latifolia 378
yeasts 1296
Waste waters
anaerobic fermentation 309
anaerobic treatment, conferences 139
aquaculture, reviews 2006
coke industry, germanium, recovery 2062
land application
reviews 1992
soil properties 1990
methane production, reviews 309
microorganisms, fishponds 1306
power generation
aquaculture 1384
shrimp culture 1384
Wastes
anaerobic treatment, fuels, production 1321
biological treatment 1966
cereals, reviews 2037
fruits, reviews 2037
land application 1311, 1988
medical, landfill 772
municipal refuse, landfill 772
vegetables, reviews 2037
Water pollution
rivers
Aeromonas hydrophilia 302
cannery effluent 302
meatworks effluent 302
potato factory effluent 302
poultry, effluents 302
Salmonella 302
sewage effluent 302

ter purification, activated carbon 1293
ter reuse, reviews 2006
ter storage, timbers, decay 632
terborne salts, wood preservatives 220
termelons, fungi, India 1640
xes
lemons, *Penicillium digitatum* 1621
tangors, *Penicillium digitatum* 1621
heat
arthropod pests, China 468
Aspergillus 958
Aspergillus candidus, drying 77
Aspergillus glaucus, drying 77
Aspergillus glaucus group 73
bibliographies 76
citrinin, estimation, liquid chromatography 431
Cryptolestes ferrugineus
cypermethrin 1548
fenvalerate 1548
malathion 1494
pirimiphos-methyl 1494
deoxynivalenol 414, 457, 480
distribution, milling 956
sodium bisulphite 1560
USA 955
Ephestia cautella
methacrifos 1264
pirimiphos-methyl 1264
Ephestia kuehniella, proteins 467
fungi, Saudi Arabia 456
Fusarium 440, 448
Poland 472
Fusarium sporotrichioides, trichothecenes 899
insect pests
control 1547
damage 57
heat treatment 466
refrigeration 1554
sampling techniques 1551
sorptive silica dusts 1558
Turkey 471
ultrasound 732
insects
carbon disulphide 746
carbon tetrachloride 746
mycotoxins 440
Poland 472
ochratoxins
Denmark 957
estimation, liquid chromatography 431
Oryzaephilus surinamensis
chlorpyrifos-methyl 921
etrimfos 921
fenitrothion 921
malathion 921
methacrifos 921
pirimiphos-methyl 921
Penicillium jenseni 958
pest control, fumigants 286
Plodia interpunctella, microbial pesticides 1493
residues, malathion 1555
Rhyzopertha dominica
control 75
damage 1512
dichlorvos 1553
ecology, population dynamics 1552
heat treatment 1557
neem extracts 284
plant extracts 1916
population dynamics 1499
uric acid 949
Sitophilus granarius 716
chlorpyrifos-methyl 921
controlled atmospheres 1556
etrimfos 921
fenitrothion 921
fungi 74
malathion 921
methacrifos 921
methyl bromide 1549
pirimiphos-methyl 921
ultrasound 733
uric acid 949
varietal susceptibility 469
Sitophilus oryzae 73
biological control 1550
biology, development 1496

Wheat *cont.*
Sitophilus oryzae cont.
control 75
damage 1512
development 924
dichlorvos 1553
fungi 74
phosphine 1559
plant extracts 1916
population dynamics 1499
uric acid 949
varietal susceptibility 950-951
Sitophilus zeamais, biology, development 1496
Sitotroga cerealella 73
control 1283
storage, equipment 927, 953
Tribolium castaneum
chlorpyrifos-methyl 921
control 75
cypermethrin 1548
development 924
dichlorvos 1553
embelin 470
etrimfos 921
fenitrothion 921
fenvalerate 1548
malathion 921
methacrifos 921
pirimiphos-methyl 921
varietal susceptibility 954
Tribolium confusum, proteins 467
trichothecin, Japan 1561
Trogoderma granarium
proteins 467
varietal susceptibility 952
Wheat bran
bakers' yeast, production 806
utilization, *Saccharomyces cerevisiae* 806
Wheat flour, *Tribolium castaneum,* growth regulators 1582
Wheat straw
toxinogenic fungi 883
utilization, *Agaricus bisporus* 321
Whey
acetones, production 2040
anaerobic digestion 765, 2051-2052
antibiotics, production 2046
biomass accumulation 1378
butanol, production 1368, 2040
citric acid, production 2043
edible fungi, production 2046
enzymes, production 1361
ethanol
production 1362, 1365, 1367, 1372, 2042, 2045-2048, 2050
economics 2044
feeds, production 2033, 2043
fermentation 1321
Candida curvata 363, 1370
Candida pseudotropicalis 1365, 2049-2050
Clostridium acetobutylicum 1374-1375
Kluyveromyces fragilis 1362, 1365, 1373, 2047, 2049-2050
Kluyveromyces lactis 1378
Kluyveromyces marxianus 2048
Lactobacillus casei, patents 810
Propionibacterium acidi 775
Propionibacterium shermanii, patents 810
propionic acid bacteria 2033
Saccharomyces cerevisiae 1372, 1377, 2047
Saccharomyces fragilis 2049-2050
Trichosporon cutaneum 1376
Xanthomonas campestris 1379
fuels, production 1321
gums, production 1379
lactic acid, production 1369, 2045
lactose, hydrolysis, *Kluyveromyces bulgaricus* 809
methane production 765, 2051-2052
oils, production 1370
propionic acid, production 2041
protein concentrates, upgrading, *Kluyveromyces fragilis* 774
proteins, production 1363, 1996, 2050

Whey *cont.*
single cell proteins, production 1364, 1376-1377, 2049
solvents, production 1374-1375
ultrafiltration permeate
butanediol, production 776
utilization, *Klebsiella pneumoniae* 776
utilization
Aspergillus niger 2043
basidiomycetes 1363
biotechnology 2045
Canada 2046
Candida pseudotropicalis 1364
Clostridium acetobutylicum 2040
Clostridium beyerinckii 1368
Enterobacter cloacae 1366
Kluyveromyces 2046
Kluyveromyces fragilis 1361, 1364, 1367, 1996, 2042
Kluyveromyces lactis 1364
Lactobacillus bulgaricus 1369
Morchella crassipes 2046
Propionibacterium rubrum 2041
Saccharomyces anamensis 370
Saccharomyces cerevisiae 1367
genetic engineering 2053
thermophilic bacteria 2042
Torulopsis candida 1364
vitamin B12, production 2033
Whipping cream
endotoxins, Limulus test 1678
psychrotrophic bacteria, sensory evaluation 1677
Whisky, wastes, land application 1311, 1988
Windows
fungi, Finland 241
preservation 1809
Wine industry, waste water treatment, reviews 2039
Wines
bacteria, sorbic acid 991
palms, preservation 98
trichothecin, detection 992
yeasts, sorbic acid 991
Wood
Agaricales, reviews 651
basidiomycetes 646
biodegradation 841
biodeterioration, soils 646
decay, techniques 1913-1914
decay fungi
wood anatomy 641
wood chemistry 641
fungal decay 652
Hypholoma fasciculare, growth 1173
Ischnoderma resinosum, enzyme activity 653
Phanerochaete velutina, growth 1173
Phellinus tremulae, Canada 634
Poria placenta, wood anatomy 1746
Rhodotorula nothofagi 208
Wood borers
control, HCN 239
logs
Canada 1180-1181
control 225
timbers
control 199, 218, 1179
Poland 659
slaked lime 221
Wood chemistry, biodegradation, books 841
Wood chips
fungi 207
storage 1170
Inonotus weirii 1270
stain fungi, pulping 1813
thermophilic actinomycetes, storage 1170
Wood destroying fungi, biodeterioration, reviews 1756
Wood preservation
bibliographies 676
Botswana 1187
building timbers 1189
buildings 666
Tanzania 1737
Canada 1804
conferences 826, 1397, 2077-2078

Wood preservation *cont.*
field tests 1196
Fiji 666
fungi
laboratory tests 1912
timbers 205
glossaries 1188
hives 1792
incising 1776, 1798
India 1779
laminated wood 1797
legislation, safety 1789
Lesotho 1187
marine structures 222
methodology 663
Switzerland 1794
Mozambique 1187
Namibia 1187
New Zealand
marketing 1783
standards 1190
organizations, Europe 1183
particleboards 662
plywood 662
poles 1163
Isoptera 1773
posts 1191
pressure treatments 1189
quality controls, USA 1192
reviews 229, 1782, 1803
Scandinavia 1186
South Africa 1187
standards 1188
German Federal Republic 670
Taiwan 1805
USSR 1781
Swaziland 1187
testing
basidiomycetes 1193
statistical methods 1197-1198
strength 1194
thatch 666
timbers 662
Isoptera 658
methodology 226
Tropics, books 1401
Zimbabwe 1187
Wood preservatives
alkylammonium compounds 1800
ammonium compounds 1208, 1417, 1773, 1801
As compounds 1807
azaconazole 1786
B compounds 662
Basilit 1206
borax 1206, 1814
boric acid 1814
Bulgaria 1777
carbamates 1804
carbendazim 1804
carbolineum 674
chemical analysis 1415
chlorinated hydrocarbons 1793, 1810
chloropicrin 223
copper-8-quinolinolate 1812
Cr-As-F 1795
Cr compounds 1807
Cr-F 1795
creosotes 220, 227-228, 661, 671-672, 674, 1412, 1419-1422, 1773, 1776, 1784, 1788, 1795, 1802, 1912
Cu-As 1417
Cu-As-F-phenol 1801
Cu-B-F 637
Cu compounds 662, 1191, 1800
Cu-Cr 1795, 1912
Cu-Cr-As 662, 665, 1184, 1191, 1210, 1413, 1759, 1773, 1776, 1795, 1798, 1800, 1811
retention 669
tannins 1799
Cu-Cr-B 1795
Cu-Cr-B-F 1208
Cu-Cr-F 1185
cuprammonium caprylate 1811
cypermethrin 660
dichlorophen, analysis 231
dichlorvos 659
dinitrobutylphenol 1184
endosulfan 659
Wood preservatives *cont.*
3-ethoxycarbonyl oxyl-1-bromo-1,2-diiodpropene 1812
F compounds 675, 1749, 1790, 1801
industrial wastes 1201
fenitrothion 659
fumigants 1796
fungicides, testing 1418
furcarbanil 1749
halogenated hydrocarbons 664
HCN 239
lindane 659
metallic naphthenates 1812
methoxychlor 659
methyl bromide 1774
methyl isothiocyanate 1203
methylene bisthiocyanate 1804
oil-borne compounds 671-672, 1411, 1421, 1802
oil-borne solvents 1416
organic solvents 1189
organoiodine compounds 1812
organotin compounds 220, 1812
PCP 663, 1196, 1414, 1773, 1793, 1795
PCP-Na 663, 1166, 1199, 1206, 1804, 1814
penetration 1806
pentachlorophenyl laurate 1812
permethrin 662
petroleum oils 1200
phenolic compounds 1793
pyrethrins 1786
resins 1778
retention 1207
S compounds 668
sodium carbonate 1199
sodium pentachlorophenoxide 232
standards
German Federal Republic 1204
USA 1411-1422
sulphenamides 668
tars 661, 1412, 1420, 1801
TCMTB 637, 1804
TCP 205, 1810
thiabendazole 1810
thiometon 659
tribromophenol 1812
tributyltin oxide 230, 662, 1948
waterborne salts 220
Zn compounds 1778, 1807, 1812
Wool textiles
Anthrenus flavipes, fenvalerate 679
Attagenus scalaris 270
Attagenus unicolor, fenvalerate 679
insect pests, permethrin 1816
mothproofing 679
Tineola bisselliella, fenvalerate 679
Wraps, aubergines 132
Xanthomonas campestris, whey, fermentation 1379
XB-5153, bacteria 1228
Xylan
biodegradation 789
Cellulomonas uda 1338
Clostridium acetobutylicum 793
Cryptococcus 2019
Pichia stipitis 2019
yeasts 2019
ethanol, production 2019
D-xylan
biodegradation
Thermomonospora curvata 2013
Thermomonospora fusca 2013
Xylanase
activity, *Cellulomonas* 338
production, *Melanocarpus albomyces,* bagasse 818
Xylanolytic microorganisms, *Aspergillus ochraceus* 2070
Xylaria, timbers 633
Xylaria hypoxylon, Fagus 649-650
Xyleborus similis, timbers, control 1766
Xylobolus frustulatus
lignin 209
ligninolytic microorganisms 209
Xylose
ethanol, production 2068
fermentation, yeasts 2068
D-xylose
utilization, *Pachysolen tannophilus* 1332
biodegradation, *Pachysolen tannophi* 2003
ethanol, production 2003
fermentation
Clavispora 794
yeasts 365
pulp mill effluent, fermentation 365
Xyloterus lineatus
timbers
lime (mineral) 1772
slaked lime 221
Xylothrips flavipes, timbers, South Afr interceptions 211
Yams
Botryodiplodia theobromae
enzymes 531
physiology 532
fungi, enzymes 134
insect pests, Ivory Coast 1647
Penicillium sclerotigenum, enzymes 135
Yeasts
activated sludge, lysis 1300-1303
beverages, techniques, detection 491
control, preservatives 25
estimation, ATP, measurement 22
foods, culture media 23
milk
infrared radiation 1705
ultraviolet radiation 1705
milk products, Egypt 1094
quarg, detection 1093
waste water treatment 1296
wines, sorbic acid 991
xylan, biodegradation 2019
xylose, fermentation 2068
D-xylose, fermentation 365
Yersina, shrimps, indoles 151
Yersinia
foods 850
culture media 857
France 857
Netherlands 1431
meat, isolation technique 543
milk
classification 1231
culture media 1709
Yersinia enterocolitica
beef
acetic acid 1066
formic acid 1066
cheeses
acidity 625
temperature 625
food poisoning 1425
foods
detection 1429
France 857
milk
Bulgaria 1132
enterotoxins 870
France 1706
virulence 697
pork 857
inhibition, microflora 1669
virulence 697
vegetables, virulence 697
Yoghurt
endotoxins, Limulus test 1678
Enterobacteriaceae, Egypt 1095
microbial flora, Iraq 1672
Salmonella, survival 176
Yugoslavia, beans, mycotoxins 479
Zabrotes subfasciatus
beans
biology 1859
damage 1571
beans (*Phaseolus*), resistance 960
control, insecticides 737
Zalerion maritimum, beech 652
Zambia, Scolytidae, distribution 264
Zimbabwe, wood preservation 1187
Zineb, tomatoes, *Cladosporium oxyspor* 1644
Zn compounds, wood preservatives 1778, 1807, 1812
Zygosaccharomyces bailii, control, preservatives 25

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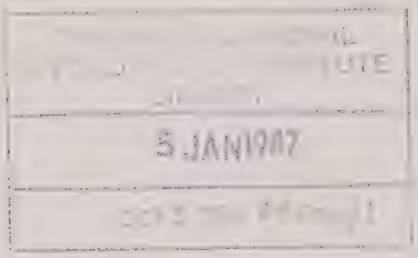

SPOILAGE AND MYCOTOXINS OF CEREALS AND OTHER STORED PRODUCTS

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FOREWORD

The invited papers collected together here form the second in a series of publications of the proceedings of the scientific meetings of the Biodeterioration Society. As with the first in the series – *Biodeterioration and Biodegradation of Plastics and Polymers,* edited by K.J. Seal – the aim has been to provide a record of the meeting as rapidly as possible.

In the papers the aim has been not only to review a range of aspects of stored products biology but also to give some indication of the current work of the authors. The emphasis is, of course, on spoilage of cereals and the papers have been arranged in four sections, dealing with insect and mite pests, microorganisms, mycotoxins and the application of newer techniques to the study of spoilage. Naturally, any meeting on stored products held in UK is bound to have a major input from both the MAFF Slough Laboratory and TDRI, and the Society is indebted to these establishments for providing more than half the speakers. The fact that more than half the papers deal with mycotoxins reflects the research effort expended in the UK on these fungal metabolites, albeit that one paper questions the justification for the attention given to mycotoxins in recent years!

I join the Editor in thanking the speakers for the prompt production of their manuscripts, in additon to presenting their papers so ably at the meeting. As the inclusion of a cartoon in the Proceedings indicates, this was not without its moments of humour! All seventy participants are to be thanked for their part in lively discussion at this stimulating and rewarding meeting. Particular thanks are due to Dr D.A. Griffiths of MAFF Slough Laboratory (who also acted as a session chairman) Mr D.R. Wilkin and Dr K. Jewers for assistance in arranging the programme; Margaret Flannigan for editorial assistance; and, especially, Dr M.O. Moss for the excellent local organisation, and also acting as a session chairman.

I wish to express my sincere thanks to Dr Brian Flannigan for his editorship of these proceedings and for acting as session chairman. I am especially grateful for his efficient overall organisation of the meeting.

Glyn Morton
President
June 1986

CONTENTS

PESTS IN STORED CEREALS

Stored grain pests and their management
D. R. WILKIN & E. T. HURLOCK .....

New approaches to the detection of pests in grain
D. B. PINNIGER, J. CHAMBERS & P. M. COGAN .....

Pest control by cooling and ambient air drying
D. M. ARMITAGE .....

New developments in pest control
D. G. ROWLANDS & J. P. EDWARDS .....

MICROORGANISMS IN CEREALS AND OTHER STORED PRODUCTS

Factors affecting fungal colonization of grain
J. LACEY .....

The microbial content of wheat and flour
D. A. L. SEILER .....

Bacterial and mould spoilage of coconut
JUDITH L. KINDERLERER .....

MYCOTOXINS IN CEREALS AND OTHER STORED PRODUCTS

Interaction between toxic and non-toxic fungi in gamma radiation sterilised cereals
J. E. SMITH & R. G. CUERO .....

Moulds and mycotoxins in cereal and other components of compound animal feeds
A. E. BUCKLE .....

Fusarium mycotoxins in cereals and other stored products
M. J. SHEPHERD & J. GILBERT .....

Natural occurrence of fungal naphthoquinones in cereals and animal feedstuffs
K. A. SCUDAMORE, J. H. CLARKE & PAULINE M. ATKIN .....

Problems involved in the determination of aflatoxin levels in bulk commodities
K. JEWERS, R. D. COKER, G. BLUNDEN, MARY J. JAZWINSKI & ANGELA J. SHARKEY .....

Efforts to control the levels of aflatoxin in South-East Asian maize
B. D. JONES, SUE KENNEFORD, M. J. NAGLER, J. MEADLEY & DARA BUANGSUWAN .....

e possible effect of aflatoxin contamination on the marketing of palm kernels from West Africa
J. CORNELIUS & E. N. MADUAGWU 95

e treatment of aflatoxin contaminated commodities
R. D. COKER, K. JEWERS & B. D. JONES 103

cotoxins and the fermentation industries
B. FLANNIGAN 109

CHNIQUES FOR INVESTIGATION OF STORED PRODUCTS

w developments in the accurate identification of aspergilli in stored products
ZOFIA KOZAKIEWICZ-LAWRENCE 115

munological detection of fungi in plants, including stored cereals
J. H. CLARKE, A. D. MACNICOLL & JOANNA A. NORMAN 123

munoassay of mycotoxins
HEATHER A. KEMP & M. R. A. MORGAN 131

totoxicity testing for mycotoxins
KATE MACKENZIE & JEAN ROBB 135

croorganisms and microbiological rapid methods in the bioassay of mycotoxins
M. O. MOSS & G. K. ADAK 141

Spoilage and Mycotoxins of Cereals and other Stored Products.
Edited by B. Flannigan

PESTS IN STORED CEREALS

Stored Grain Pests and Their Management

D. R. Wilkin and E. T. Hurlock

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Summary

Large changes have occurred in the United Kingdom cereal industry over the past 10 years. These include doubling of production, large bulk storage, greater utilisation of home-grown wheat in bread and large exports of grain. As a result the importance of pests has also increased.

Monitoring has shown that the major pests remain but that new problems have emerged. Detection of pests is extremely difficult and conventional techniques need improvement.

Grain pests are susceptible to changes in their environment and this can be exploited, provided a detailed knowledge of their biology is available. Given this, the temperature and moisture content of the grain can be controlled at levels that prevent pests breeding.

Physical methods must be supplemented by chemical control and several pesticides are available which combine low mammalian toxicity with a broad spectrum of activity against insects and mites. Some pesticides have long residual lives and can be used to protect grain for many months. Alternatively, fumigants can be used to disinfest grain and prevent damage.

Current pest management strategies are effective, but more stringent quality standards, the establishment of new pests and resistance to pesticides could lead to their breakdown. More research is needed to develop new strategies.

I. Introduction

The cereal industry in the United Kingdom has undergone major changes over the last 10 years largely as a consequence of Britain's membership of the European Economic Community. Total production and the value of the crop have more than doubled (Table 1). Britain has exceeded self-sufficiency levels for cereals and now produces an exportable surplus of between 6-10 million tonnes. There have also been important changes in patterns of usage for home-grown grain and at least 70% is now not used on the farm of origin. Virtually all trading contracts specify a moisture content of 16% or less with the result that the majority of the grain harvest is dried to below this level before storage. This reduces the potential for fungal growth so that the most commercially important agents of biodeterioration during storage are invertebrate pests. Another significant change is the rise in the percentage of UK wheat used to produce flour for breadmaking. In 1975 only about 40% of wheat was used in bread flour but this has now reached 78%.

These developments in the industry have brought about a major change in the attitude towards pest control. A number of insects and mites can attack grain during storage and cause damage such

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Table 1. *Changes in production and export of cereals in the UK over the past 10 years.*

	1975/6	1984/5
Production (10^3 tonnes)	13,796	26,528
Exports (10^3 tonnes)	1,200	7,936

Source: Home-Grown Cereals Authority, Cereals Statistics.

as weight loss, reduced germination and a fall in food value. The expansion in the industry has led t heightened awareness of pest problems and a much greater interest in storage strategies aimed preventing infestation. New standards have been applied to grain sold to some markets so t complete freedom from pests is required. Currently, up to half the UK production has to meet suc standard en route between producer and consumer. The financial penalties of even minor infestati are serious and many account for a loss of £5-15 per tonne.

The importance of the continued export of grain to avoid the accumulation of costly a wasteful surpluses elevates pest control in stored grain to a level of national importance. Hence th is a great need to develop sound storage strategies and continue to monitor problems and deve improved techniques as required.

III. Pest monitoring

MAFF staff have been monitoring the occurrence and distribution of pests for many years. The d that has been collected can be used to show trends in infestation and how pests have led to proble for the cereal industry. Its collection has largely been as a result of advisory work and statut inspection of grain exports.

Data collected from advisory visits cannot provide unbiased information but it does give an going picture of the problems that are considered serious by producers and storers of grain. Most the visits were made to farm grain stores and large commercial premises. More than 20 species h been discovered infesting grain but six species and two species of mites occurred most frequently a in the greatest numbers. Over the past six years pest monitoring data has been stored on a compu enabling annual analysis to be made and the occurrence of the major pest species between 1982 a 1985 is summarised in Table 2. Acknowledged grain pests such as the Saw-toothed Grain Bee (*Oryzaephilus surinamensis*) and the Grain Weevil (*Sitophilus granarius*) occurred regularly b two insects normally considered to be secondary pests, the Hairy Fungus Beetle (*Typhaea stercor* and the Foreign Grain Beetle (*Ahasverus advena*), occurred at least as frequently. The frequer with which these latter species caused problems is an indication of the change in attitude that h occurred towards infestation. Neither insect is capable of completing its life cycle at grain moistu contents usual in the United Kingdom. However, they can be confused with some primary pests a are regarded as quarantine pests by some countries who import grain. Each of the two species of mi caused as many or more problems than any of the insect pests. Many of the problems that led t request for ADAS advice, originated when grain was rejected at time of sale.

Table 2. *The frequency of occurrence of the major pests in farms and commercial grain stores in the United Kingdom.*

Species of insects and mites	No. of occurrences 1982*	82/83	83/84	84/85
Cryptolestes ferrugineus	19(1)	15(5)	17(2)	68(7)
Sitophilus granarius	19(0)	19(5)	17(4)	21(0)
Oryzaephilus surinamensis	32(1)	64(5)	70(3)	114(3)
Ahasverus advena	24(2)	55(6)	62(1)	126(3)
Typhaea stercorea	16(0)	86(9)	65(1)	108(2)
Acarus siro	23(0)	98(9)	36(1)	62(2)
Glycyphagus destructor	30(4)	127(13)	48(2)	123(3)
Total no. of advisory cases on farm or in commercial stores ( )	109(20)	422(56)	282(34)	654(32)

*6 months' figures only

As stated earlier the level of grain exports from the United Kingdom has increased more than fold over the past 10 years, and exports now have a total value of about £1,000 million. Almost h the grain exported is shipped to countries that require phytosanitary inspections to check for t

presence of pests. The results from these phytosanitary inspections, which are summarized in Table 3, give a good indication of the endemic levels of infestation. Suppliers of grain for export are well aware of the consequences of insects being discovered and therefore would not knowingly offer infested grain.

Table 3. *Level of infestation detected during phytosanitary inspections of grain exported from the UK.*

Export Grain	Calendar year	"Storage" years August–July			
	1979	1981–82	1982–83	1983–84	1984–85
Total tonnage inspected	500,000	536,546	826,379	1,547,397	2,102,996
Total number shipments seen	340	346	212	284	282
Number shipments where infested grain intercepted	108	78	27	52	44
Potentially infested shipments (per cent)	31.8	22.5	12.7	18.3	15.6

The majority of insect pests that infest grain are originally of tropical origin and have been introduced into Britain. This process of introduction is ongoing. ADAS carries out limited inspections of certain food commodities that are imported into the United Kingdom to monitor the levels of infestation. The data collected, summarised in Table 4, shows that a substantial proportion of imports are still infested and that most of all our major grain pests are regularly imported. Some of the infested commodities have direct links with agriculture as they are used in the manufacture of animal feed. These can be delivered directly to farmers or be supplied to provender mills that distribute the feedingstuffs to farms. This regular importation of insects has even more serious implications for the UK grain industry as many of the imported strains have been shown to be resistant to the pesticides currently in use.

Table 4. *Infestation detected during limited monitoring of food or animal feed imported into the UK.*

	1981	1982	1983	1984	1985
No. shipments examined (freight containers and conventional cargoes):	954	1,016	558	326	297
% shipments found to be infested:	32	29	32	27	33
Principal grain pests recorded:	Rate per 100 infested shipments:				
Sitophilus granarius	1	2	1	1	1
S. oryzae	3	4	8	2	4
Ahasverus advena	7	14	17	15	8
Cryptolestes ferrugineus	8	8	3	15	14
Acarus siro	7	6	7	3	1
Glycyphagus destructor	4	4	1	1	1
	3	4	–	1	–

The biology and ecology of pests

The tropical origin of the majority of insect grain pests means that they require relatively high temperatures to breed. For example, *O. surinamensis* can only complete its life cycle at more than 18°C and has an optimum of 35°C. *S. granarius* has a lower minimum temperature of about 12°C but still requires 25°C for rapid reproduction (Howe 1965).

Grain may be harvested from the field at a temperature of 25°C or more and drying may further raise the grain temperature. Clearly, storage at 25°C or more will allow any insects present to breed. The metabolic heat from these insects is then produced more rapidly than it is lost through the grain. This can cause the temperature in the centre of a grain bulk to rise to 40°C or more and lead to widespread damage to the grain. Heavy infestations can lead to extensive damage to the grain and loss of quality (Freeman 1973, Howe 1965, Hunter 1980).

Mites can complete their life cycle at temperatures as low as 5°C so are less susceptible to cooling as a control measure. However, they do require certain minimum levels of relative humidity within the intergranular spaces which are controlled by the moisture content and temperature of the grain. The common pest species can only breed in grain with moisture contents of at least 14-15%

(Cunnington, 1976). Unfortunately, some contracts for grain specify moisture contents of up to 16%. Hence, there may be some conflict between commercial interest and good storage practice.

V. Physical control

The dependence of both insects and mites on the physical conditions within grain makes the susceptible to control by modifying their environment. However, such an approach requires detailed knowledge of the biology of the pests and a clear understanding of the physical characteri tics of grain bulks.

Drying grain immediately after harvest and cooling it in the early part of the storage period for an essential part of storage strategy. Other physical measures such as hygiene can also play a important part in preventing the development of infestations. Most grain stores are empty for at lea part of the year but pests can survive in relatively trivial quantities of grain residue. Thorou cleaning will eliminate these sources of food, making it more difficult for the pests to survive and the very least reduce the size of the population available to infest the grain when the store is refille

VI. Chemical control

Despite the obvious potential for limiting the populations of stored product pests by control of th environment, there are still many situations where some form of chemical control is required. F example, some markets both within the UK and abroad demand complete freedom from all li pests. Meeting such standards by purely physical methods can be expensive and problematic Hence, a number of control techniques have been developed using various pesticides.

Chemicals that are used to treat grain stores and grain must possess the correct blend biological activity and either low mammalian toxicity or short residual life. Currently 22 chemica are cleared by the Ministry of Agriculture, Fisheries and Food's Pesticide Safety Precaution Schem for use in grain stores or for application to grain. The chemicals and their cleared uses are sum marised in Table 5.

The pesticides fall into two principal categories; contact chemicals which are persistent to som degree and fumigants which are active in the vapour phase but are non-persistent.

Contact pesticides can be applied to the structure of the empty store to control any pes remaining after cleaning. Such treatments are effective in modern, sound structures that offer th minimum of harbourages to protect the pests. However, if the pests can escape contact with th pesticide by sheltering in deep harbourages, structural treatments cannot be guaranteed to gi complete control. Alternatively, certain contact pesticides can be added directly to the grain. Th technique can be very effective and, when combined with appropriate physical measures, will ensu grain remains free from live pests for many months. The low cost (15-40p/tonne) and effectiveness the method has led to the widespread adoption of admixture of pesticides with grain, particularly i commercial grain stores (Wilkin *et al.* 1982). The reduction in the level of infestation detected grain examined at export terminals over the past six years (Table 3) is almost certainly attributab to the effectiveness of this technique.

Fumigation is also extremely effective and provides a valuable method of dealing with esta lished infestations, thus ensuring that grain is pest-free at point of sale. In some cases this techniq may be the only applicable control strategy for rectifying serious problems and preventing pests fro causing substantial damage. However, the toxic hazards associated with the application of fumigan restrict their use to pest control contractors and also make the technique expensive (Bell & Rowlan 1983). Note that not all of these chemicals may be currently available.

VII. Future developments

The world surplus of cereals and the rapid increase in European stocks, will inevitably result in ev tightening quality standards. This will lead to greater pressures to control infestation and increase t financial penalty for failing to do so. The price of cereals is likely to remain static or even fall th reducing profit margins so that the cost effectiveness of pest control measures will become even mo important.

Current control strategies, based on physical control and the use of pesticides as required, a reasonably cost effective. Economic pressures will undoubtedly change this situation and requ further improvements, particularly in the effective use of physical control measures. Howev technical problems caused by changing patterns of storage and limitations of chemical cont demand urgent action if pest control strategies are not to break down.

The size of grain stores is increasing and it is now common to have 50,000 tonnes of grain stor as a single bulk. The detection of pests, both as the grain goes into store and during storage assur

Table 5. *Pesticides currently cleared by MAFF Pesticide Safety Precautions Scheme for use in grain stores.*

FUMIGANTS

Carbon tetrachloride
Ethylene dichloride
Methyl bromide
Phosphine

CONTACT PESTICIDES

Structure of store	Grain
Bendiocarb	Chlorpyrifos-methyl
Bioallethrin	Etrimfos
Bioresmethrin	Fenitrothion
Bromophos	Malathion
Chlorpyrifos-methyl	Methacrifos
Cypermethrin	Pirimiphos-methyl
Dichlorvos	
Etrimfos	
Finitrothion	
Gamma-HCH	
Iodofenphos	
Malathion	
Permethrin	
Pyrethrins	
Pirimiphos-methyl	
D-phenothrin	
Tetramethrin	

paramount importance in these very large bulks. Better detection techniques must be a very high priority for future research.

The admixture of pesticides with grain is a widely used and cost effective control measure. However, its continued use will entail overcoming two problems. Firstly many grain pests have

Table 6. *The incidence of resistance in insects collected in UK grain stores.*

Species	Number of strains					
	1970s			1983–85 (e)		
	Resistant	Susceptible	% Resistant	Resistant	Susceptible	% Resistant
C. ferrugineus	no resistance known		0	5	17	22.7
O. surinamensis	18	985	1.8 (a)	27	70	27.8
S. granarius	5	90	5.3 (b)	2	18	5.0
S. oryzae	1	27	3.6 (c)	0	6	0
T. castaneum	36	6	85.7 (d)	6	0	100

(a) 1973–78
(b) 1973–79
(c) 1973–79
(d) 1970–77
(e) August 1983–July 1985

developed resistance to the pesticides currently in use as grain protectants. The rise in the level of resistance in *O. surinamensis* over the past 15 years is shown in Table 6. Secondly the application of pesticides directly to grain gives rise to residues that are passed into the food chain (Wilkin & Fishwick 1981). Whilst these residues are within accepted international tolerances, strategies allowing more selective use of pesticides and methods of reducing residue levels must be sought. Future use of fumigants may also be limited by problems associated with resistance and unacceptable residues. In the longer term new approaches to the control of grain pests may be needed. New types of pesticide are required to overcome resistance. Radical techniques such as reducing oxygen levels in the intergranular spaces will be needed. All these developments will require a continued and substantial research effort if theoretical ideas are to be translated into practical reality. However, improved

storage strategies incorporating these new developments are vital if the UK cereal industry is continue to be efficient and able to export grain surpluses profitably.

References

Bell, C.H.; Rowlands, D.G. (1983) The future of liquid fumigants. *Proceedings of the Sixth Pest Control Conference, Cambridge 1983.* Sessio paper 19, 10pp.

Cunnington, A.M. (1976) The effect of physical conditions on the development and increase of some important storage mites. *Annals of App. Biology* **82**, 175-178.

Freeman, J.A. (1973) Problems of infestation by insects and mites of cereals stored in Western Europe. *Annales de Technologie Agricole* (Paris) 509-530.

Howe, R.W. (1965) A summary of estimates of optimal and minimal conditions for population increase of some stored products insects. *Journa Stored Products Research* **1**, 177-184.

Hunter, F.A. (1980) Problems encountered in protecting stored grain from damage by rodents, birds, insects and mites in England and Wa *Progress in Food and Nutrition* **4**, 79-90.

Wilkin, D.R.; Cruickshank, S.L.; Dyte, C.E. (1982) Pesticide usage on grain in commercial grain stores. *International Pest Control* **25**, 82-8

Wilkin, D.R.; Fishwick, F.B. (1981) Residues of organophosphorus pesticides in wholemeal flour and bread produced from treated wheat. *Proce ings, 1981 British Crop Protection Conference, Brighton*, 183-187.

oilage and Mycotoxins of Cereals and other Stored Products.
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New Approaches to the Detection of Pests in Grain

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Summary

Insect pests are difficult to detect in grain bulks using the conventional method of sieving samples removed from the bulk with a spear or vacuum sampler. Small numbers of insects can remain undetected in store and this may lead to development of infestation during storage and rejection of grain when it is sold.

Recent trials by Slough Laboratory staff have compared the performance of pitfall and probe traps with conventional sampling and sieving in floor-stored grain bulks. The traps were more than ten times as effective as spear or vacuum sampling in detecting low levels of infestation of our three major beetle pests of stored grain (*Oryzaephilus surinamensis*, *Sitophilus granarius* and *Cryptolestes ferrugineus*). The traps are now in use in commercial grain stores in the UK and have already shown their value by early detection of pest problems thereby reducing risks of rejection and damage to grain. Current work is aimed at improving the efficiency of the traps in static bulks possibly by the inclusion of chemical attractants.

Although traps are cheap and effective they are only suitable for static bulks of grain. A method is needed for detection of insects in grain in transit, for example in a lorry load arriving at a grain store. Tests have shown that nuclear magnetic resonance (NMR) can be used to detect those insects and their immature stages which are hidden within grains. Further development is needed but it seems likely that this and other methods based on new technology will provide the key to practical detection methods in the future.

Introduction

Successful strategies for the safe storage of bulk cereals are dependent upon effective techniques for detecting pests. Early warning of the presence of insects may enable infestations to be controlled by physical or chemical methods before serious damage has occurred. When grain is traded there is a need to detect low numbers of insects in two distinct and very different areas. The first is the monitoring of large static grain bulks for periods of a few months to several years and the second is the examination of grain in transit between storage points.

Current methods for the detection of insects in static grain bulks and in transit are based on the removal of samples by spear or vacuum and the sieving of the samples to look for insects. This process

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is laborious, time-consuming and relatively ineffective for detecting low levels of infestation of ad insects or developing stages of some insect pests.

Some warning of developing infestation can be obtained from interpretation of temperat trends in static bulk stores equipped with temperature sensors. However, this method can only g warning when infestation levels have reached an unacceptable level in a bulk and it cannot be appl to grain in transit.

Insect pests will survive in empty stores for long periods of time after the removal of infes grain. Work at the Slough Laboratory has demonstrated the value of insect traps for detection insects in empty stores and subsequently monitoring the effectiveness of residual insecticide tre ment in the store (Pinniger 1975, Pinniger *et al.* 1984). The need to develop and evaluate equival methods for the rapid detection of insects in grain is clear and the problem has been approached two ways because of the very different requirements for the successful monitoring in static bu compared with detection in grain in transit.

III. Trapping in static bulks

Improvements to the standards achieved by sampling bulks and sieving grain have been sought many countries. Pitfall traps (widely used in ecological studies of beetle distribution in woodlan etc.) have been adopted in some German grain stores and therefore it was decided to evaluate pit traps in grain bulks in the UK (Cogan *et al.* 1986). The trap used in initial trials was a 2 lb glass j jar with the inside rim coated with "Fluon". The traps were pushed into the grain until the rim flush with the surface. Insects which fell into the trap were unable to escape due to the "Flu barrier. In all later trials the glass jars were replaced with 1 pt clear polystyrene beer glasses to av possible hazards from broken glass.

A probe trap based on pitfall principles was developed in Canada by Loschiavo & Atkins (1973). After further development in the USA (Barak & Harein 1982, Wright & Mills 1984) it eventually marketed by Zoecon Industries. A probe trap is a clear perspex cylindrical tube 36 c 2.5 cm with 186 3-mm dia holes drilled at an angle of 60°. A removable collection tube is house the bottom of the trap. The probe is pushed vertically into the grain until the top is just below surface.

It was decided to compare the performance of pitfall and probe traps with the establis conventional sampling and sieving technique in laboratory tests and field trials.

IV. Laboratory tests

The experiments were carried out in circular 230 litre (50 gall.) plastic bins, approx. 75 cm high x cm diameter. These were filled with 150 kg of barley from which dust and fine material had b removed by sieving. The top inside rim of each bin was coated with Fluon to prevent the inse escape. The temperatures varied between 16.4°C and 24.3°C. Humidity was not controlled measured. The traps were tested separately against three insect species, *Oryzaephilus surinamen Sitophilus granarius* and *Cryptolestes ferrugineus* at a density of 1 adult insect/kg (150 per b

The insects were introduced to the centre of each bin and left for 3 days to disperse and set Pitfall traps and probe traps were then put in the centre of the appropriate bins and the numbe insects in each trap was counted after three days. A single 0.3 kg spear sample was taken just be the grain surface from separate bins and the sample was sieved over a 10 mesh sieve to remove ins for counting. The results given in Table 1 show the total number of each insect species caught by three methods.

Table 1. *Summary of Laboratory Trials.*

	Spear	Pitfall	Probe
Oryzaephilus surinamensis	2	40	9
Sitophilus granarius	9	136	14
Cryptolestes ferrugineus	2	1	19

Total number of insects from 5 replicates

The numbers of *O. surinamensis* and *C. ferrugineus* in the spear samples were close to th expected, assuming a random distribution of insects in the grain.

The highest numbers of *O. surinamensis* and *S. granarius* were found in the pitfall traps and highest numbers of *C. ferrugineus* were in the probe traps. The catch of insects is influenced by t design and insect behaviour: in this series of experiments it was noticed that *S. granarius* adults w seen frequently on the grain surface whereas *C. ferrugineus* adults were never observed. The res from the traps probably reflect the trap positioning in relation to the wandering activity of the inse

It was decided to deploy both pitfall and probe traps in commercial grain stores to establish whether the supremacy of the traps over spearing in controlled laboratory experiments could be confirmed under practical storage conditions.

Field trials

Four bulks of floor-stored wheat ranging from 4,000 to 17,000 tonnes and one 7,500 tonne bulk of barley were monitored using pitfall and probe traps and the results compared to the detection rate of insects using conventional sieving of spear samples. A summary of the results over a 6-month period is given in Table 2. At all these sites the grain was thought by the storekeepers to be free of infestation.

Table 2. *Grain store trials.*

	Total number of beetles recorded		
	Spear	Pitfall	Probe
Oryzaephilus surinamensis	3	127	30
Sitophilus granarius	0	8	13
Cryptolestes ferrugineus	0	23	136

The results from these five grain stores provided emphatic evidence that the two trap types were catching insects when infestation would have remained undetected by conventional sampling techniques. As the laboratory trial showed that pitfall traps were effective for *Sitophilus granarius*, the low figures in the field trials probably reflect the low incidence of this beetle in the trial sites, as it was only found in two of the stores. The ability of the probe traps to detect *C. ferrugineus* is notable as this species was found in much smaller numbers in pitfalls and never found in spear samples. It is worth remembering that the probe used in these trials was base on a design specifically devised to catch *Cryptolestes* sp. in Canada.

Further evidence of the value of the two trapping methods were provided from a store in Suffolk where a 4,000 tonne bulk of wheat was continuously monitored for 14 months (Table 3).

Table 3. *Total numbers of beetles recorded in a wheat store*

	Spear	Pitfall	Probe
Oryzaephilus surinamensis	0	3	13
Sitophilus granarius	0	49	25
Cryptolestes ferrugineus	0	2	1

Despite intensive sampling at this store by the staff, insects were not detected, other than in traps, until the grain was being moved and an infestation of *O. surinamensis* and *S. granarius* was discovered.

The results of the laboratory experiments and practical trials have been used to formulate recommendations to the grain trade for the use of pitfall and probe traps. These two trap types used together in a store should increase the chances of detecting insects by at least 10 times when compared to existing methods. Even though the technique is relatively new, a number of commercial stores have already avoided infestation problems because of the early warning provided by successful deployment of traps.

Current research is aimed at enhancement of the trapping performance of pitfall and probe traps by addition of pheromone or food attractant lures. More effective traps will result in more efficient detection of insects in static bulks and this should lead to more effective pest control strategies and the possibility of reduced pesticide use.

. Grain in transit

Trapping methods are relatively cheap and with the improvements described above are also quite effective, but they are suitable only for static bulks of stored grain. However good the method, time still has to be allowed for the insects to be trapped and the traps inspected : this may not be possible with grain in transit, for example in a lorry arriving at a grain store or in a ship waiting to unload. Furthermore, in the case of insects such as the grain weevil, *S. granarius*, which develops unseen within the wheat kernels, trapping methods can detect only the emerged adults : by the time these have been found an infestation of this important pest may have become well established. Thus there is a need for a detection method which is rapid and does not miss insects within individual kernels.

These criteria are exacting and although no such method currently exists there are several physical techniques, including near infra-red reflectance (NIR), nuclear magnetic resonance (NMR) and acoustic recording, which might provide the basis for a suitable method. Our work with NMR illustrates the progress and problems involved in such development (Chambers *et al.* 1984).

NMR spectrometers are common equipment in organic chemical laboratories where they are used to study the molecular structure of liquids or solutions of solids. The feature of the NMR spectrometer which makes it promising for use as a detection method is that it is capable of measuring liquids, which give tall narrow peaks, in the presence of a vast excess of solids, which give only a broad shallow peak. This is precisely the situation to be found when insects occur in bulks of grain. The insects can be regarded as tiny packets of liquid, indeed they depend for their survival on their ability to retain water in the relatively dry bulk of grain.

Initial experiments were conducted on a standard NMR spectrometer. Although capable of scanning only one grain at a time it confirmed that there is a marked difference between an uninfested wheat grain, which gives only a shallow absorption, and infested grains which typically give two sharp peaks, a large one due to water and a smaller one due to lipid (Fig. 1). Of the several hundred individual grains examined to date, the NMR spectrometer has determined in a matter of seconds whether or not they are infested and has been accurate in every case. The method has been found to be equally successful for detecting the Angoumois grain moth, *Sitotroga cerealella*, in sorghum and would presumably work for any other insect in any other seed. There is however a limitation, as the size of the NMR response is proportional to the amount of liquid, so to be observable the insects must have grown larger than a certain minimum size. In the case quoted above the insects were either well developed larvae or pupae or adults.

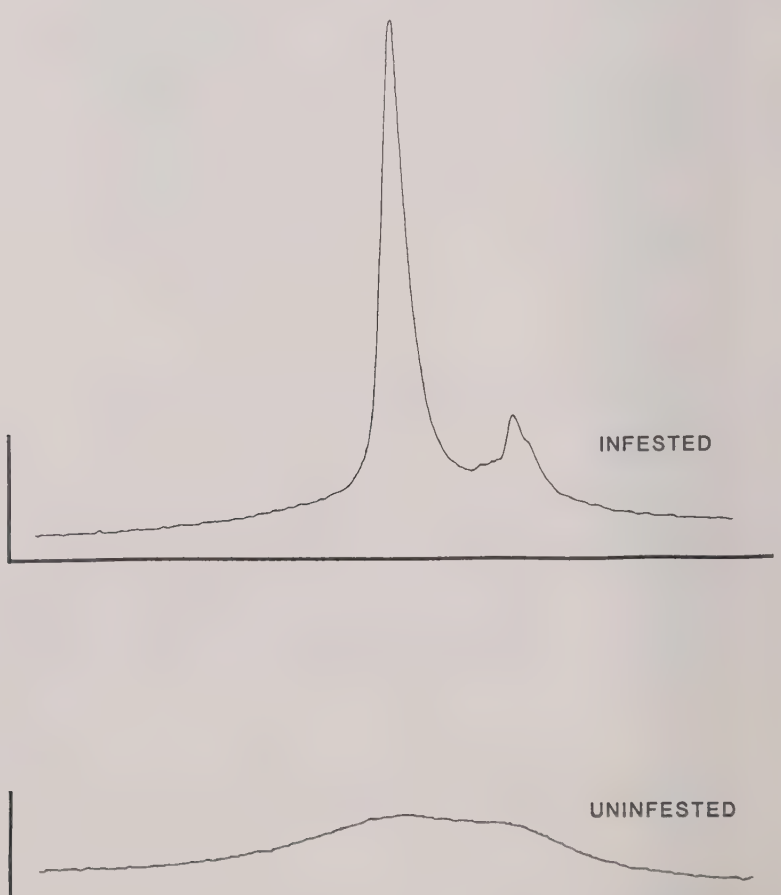

Fig.1. Comparison between NMR spectra of 2 individual wheat grains one of which is infested with a grain weevil larva.

To determine the minimum observable size (age) for *S. granarius*, thirty wheat grains in which a single egg had been laid were studied daily until adult emergence. The changes in NMR response throughout the developmental period were observed to be similar for most grains (Fig. 2). The pattern observed can be readily interpreted in terms of the water content of the insect in the various stages of its development. It is seen that the minimum observable age is about 15 days after egg

laying, when the majority of the insects would have been in the third larval instar. Although this is not ideal, it is obviously a significant improvement on the 45 or so days before the developing insect emerges from the grain and becomes detectable by sieving or trapping.

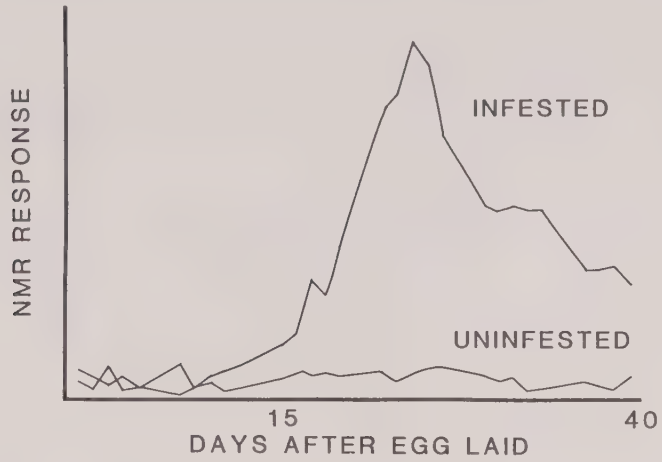

Fig. 2. Comparison between NMR response from an uninfested individual wheat grain and one infested with a grain weevil throughout the developmental period of the insect.

With a few infested grains the pattern was followed only part way, then over the next two days the NMR response decayed to finish below the level seen with uninfested grains. In each of these cases the insect was found to have died at the point at which the response began to decay. Subsequently the water in the insect diffused out of the grain leaving only the broad response of the partially eaten grain. Thus the NMR method would not be able to detect dead insects. Despite its limitations the NMR method was considered to have sufficient promise to warrant further investigation in a machine capable of examining larger samples of up to 500 wheat grains in a few seconds. An example of such a machine is the Newport Analyser which has been used elsewhere in the agricultural industry for the determination of oil in rapeseed. The results of a preliminary study show that scanning small batches of wheat grains does allow grains infested with large larvae of *S. granarius* to be detected (Fig. 3). However, the minimum level of infestation detectable with present instrumentation is about 10 insects in 500 grains, equivalent to approximately 400 insects per kilo, and this is unacceptably high.

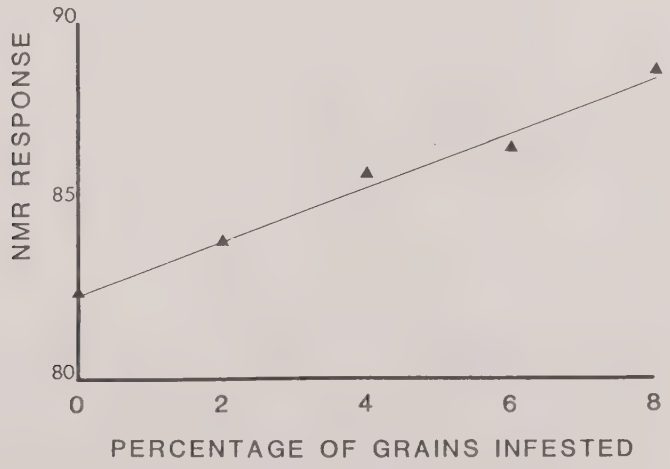

Fig. 3. NMR response obtained with a low-resolution NMR Analyser from batches of 500 wheat grains

It is hoped to undertake further investigations with instrumentation modified to permit detection of smaller or younger insects and much lower levels of infestation. If this were possible then development of a flow sampling system with diverter for samples found to be infested would allow the process to be totally automated.

Thus technically it may well be possible to develop a new detection method which would satis all of the stated criteria, but whether it should be based upon NMR or some other technique wi depend upon the success of further development.

References

Barak, A.V.; Harein, P.K. (1982) Trap detection of stored grain insects in farm-stored shell corn. *Journal of Economic Entomology* **75**, 108-1

Chambers, J., McKevitt, N.J.; Stubbs, M.R. (1984) Nuclear Magnetic Resonance spectroscopy for studying the development and detection of th grain weevil, *Sitophilus granarius* (L.), within wheat kernels. *Bulletin of Entomological Research* **74**, 707-72

Cogan, P.M.; Stubbs, M.R.; Wakefield, M.E. (1986) A laboratory and field comparison of trapping and sampling techniques for insects in grain (i press).

Loschiavo, S.R.; Atkinson, J.M. (1973) An improved trap to detect beetles in stored grain. *Canadian Entomology* **105**, 437-440

Pinniger, D.B. (1975) An assessment of residual insecticide treatments for the control of the saw-toothed grain beetle *Oryzaephilus surinamensis* i farm grain stores. *Proceedings, Eighth Insect and Fungi Conference*, 365-372.

Pinniger, D.B.; Stubbs, M.R.; Chambers, J. (1984) The evaluation of some food attractants for the detection of *Oryzaephilus surinamensis* and oth storage pests. *Proceedings, Third International Conference of Stored Products Entomology (Kansas)*, 640-65

Wright, V.F.; Mills, R.B. (1984) Estimation of stored product insect populations in small bins using two sampling techniques. *Proceedings, Thir International Conference of Stored Products Entomology (Kansas)*, 672-679.

oilage and Mycotoxins of Cereals and other Stored Products.
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Pest Control by Cooling and Ambient Air Drying

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Summary

Cooling grain by aeration to control pests is a very versatile treatment. Temperature gradients are evened out, eliminating moisture movement. Low temperatures are effective in holding both insects and mites in check, whether they are susceptible or resistant to a particular pesticide. The method is well established and has evolved with changing grain storage practice.

Aeration or cooling will not reduce grain moisture content. This can be achieved by using the ambient air or bulk drying technique. The time available for drying grain is limited by the rate of fungal growth. As currently defined the safe drying period is that prior to the first appearance of visible fungi. This criterion is variable and relevant information scarce. Thus further research is required to provide modelling engineers with an adequate biological basis for the ambient air drying technique.

. Introduction

Freshly harvested grain is usually damp enough to permit the rapid growth of fungi and mites, both of which cause heating. However, the excess moisture can be removed either in a few hours by high temperature driers, or by passing ambient air through bulks of grain over a period of several weeks. The former method involves an initial high capital investment and high running costs, so that about half of the country's grain is dried by the latter process.

After high temperature drying, the grain will be warm and so, to prevent moisture translocation which leads to fungal growth and the increase of insects and mites, the temperature of the grain has to be lowered and equalized. Continuous driers often have fans to cool the recently dried grain, but these are rarely adequate,so further cooling is needed. Both the ambient air drying technique and that of cooling involve the same principle, forcing ambient air upwards or downwards through bulks of grain. However, the techniques are not interchangeable because the volume of air needed for drying is at least 10x that required for cooling. Thus drying systems need larger fans to deliver the air and ducts of greater diameter to carry the increased volume of air. If ambient drying systems are used to cool the grain in winter, it is likely to become dampened, so the airflow must be reduced. Conversely, if large fans are attached to cooling systems in an attempt to dry the grain, the increased resistance of

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the small ducts and grain at high airflows will reduce the volume of air delivered by the fans. Coolir systems thus cannot be used to reduce grain moisture content.

III. Aeration or cooling

1. History

Prior to 1940, grain was often turned between bins, with conveying equipment, to cool it slightly ar delay the onset of pest problems. In fact, this probably served only to spread infestations and w expensive in wear and tear of machinery and in use of energy. In the 1940s, the first practical aeratic or cooling systems evolved in the USA, although the principle had been explored in the 18th centu and even in Roman granaries. The American work concentrated on combinations of fan power ar duct design necessary to cool grain quickly to prevent moisture movement (Holman 1960). The fir major biological input to the subject was made in Britain by Burgess and Burrell (1964). Th suggested a temperature at which grain was safe from insect invasion, defined conditions of moistu content and temperature for 'safe' storage, tested if the technique was applicable to the Briti climate by examination of metereological records, then proved its application in a series of large sca experiments. Their recommendations were qualified in the detailed discussion which also foresha owed future developments. The main recommendations may be summarised as follows:-

1. Cool the grain to 17°C.
2. Do not aerate if the r.h. of the ambient air exceeds 70-80%.
3. Below 10°C, grain can be stored above 17% moisture content (m.c.).

Today, these recommendations are still the basis of most advice; however, changing practices ar recent research show that they need to be treated with caution.

IV. Changing practices

It was originally suggested that grain be cooled to 17°C, because the reputedly most cold resistant the common beetle pests, *Sitophilus granarius* needed more than 100 days to complete its li cycle at this temperature (Eastham & Seagrove 1947). Today, grain is stored for much long periods, so the recommended temperature must be below that at which insects can breed. In additio the trade will not tolerate live insects and sets a limit on mite numbers; so lower temperatures to k insects and combat mites, are desirable.

Initially it was also recommended that to prevent increases in moisture content only dry a should be used to cool grain; today we know that the slight temperature rise of an aeration fan lowe the r.h. of the air, which is one of the advantages of blowing over suction systems. The old recomme dations restricted aeration hours and limited the efficiency of the technique.

The temptation of storing damp cereals to save drying costs or for other reasons, such temporary pre-drier storage, minimising dust problems during conveying or increasing palatability stock, was balanced against the inevitable slow mite and fungal growth (Hyde & Burrell 197 Today, the health hazards of mites and fungi - nutritional problems for stock, allergic reactions a mycotoxins - are unacceptable and the cool storage of grain in equilibrium with a r.h. above 70% c no longer be confidently recommended.

V. Insect control - selection of grain temperature

Grain should obviously be stored below a temperature at which insects can breed. A lower limit of 1 15°C is reportedly the lower limit of our most cold hardy pest, *S. granarius* (Howe 1965) and wou therefore appear to suffice. Clearly, the temperatures must be achieved before the insects have chance to build up their numbers, so the rate of airflow has been selected to ensure sufficiently sw cooling.

There are good reasons for reducing grain temperature below 12°C. If the grain is infested t pests may survive long periods at 12°C and will eventually rewarm the grain. It has been said that 9°C, infestations will be unable to re-heat the grain (Hyde & Burrell 1969). However, in prolong storage the grain will gradually warm in spring, enabling infestations, even those at one time cool below 9°C, to heat again. It is therefore not sufficient merely to achieve a temperature of 9° Continued intermittent aeration is necessary to maintain that temperature. In fact, by 1973 (Hyde Burrell) it was stated that temperatures below 5°C could be achieved in 2-3 months and that ze temperatures were common. The cost of the few extra fan hours required to achieve freezi temperatures is low and the advantage of keeping the grain at such temperatures, preventing mi development and eliminating insects, is obvious.

. The survival of insects at low temperatures

Published work suggests that all *S. granarius* would die between 22 (Robinson 1928) and 90 days (Mathlein 1961) and *Oryzaephilus surinamensis* between 6 (Obretenchev 1983) and 25 days (Cotton 1956) at 0°C. It seems possible therefore that insects in grain cooled to near freezing might be killed before the grain was resold. However, these optimistic forecasts must be viewed with some caution as a number of factors can affect the survival of insects in cooled grain. Acclimatisation is the most important (Ushatinskaya 1948, Evans 1981), but strain (David *et al.* 1977), r.h. (Ushatinskaya 1950) and the presence of food as broken rather than intact grain (Granovsky & Mills 1982) can all increase survival time. Because of these uncertainties, recent research at Slough has concentrated on the survival of insects, both in the fluctuating conditions that exist in grain stores and also under constant laboratory conditions. The adult beetle is the stage most resistant to low temperatures (Obretenchev 1983), so research has concentrated on adults.

During the winter, grain at the surface of aerated bins fluctuates in temperature with ambient and also gains moisture. That at the centre cools to about 0°C and both temperature and humidity remain stable. Thus insects at the grain surface have to survive lower temperatures than at the centre (although maintained for short periods), but they also experience milder temperatures and higher moisture contents at other times, allowing them to feed and repair internal damage. Any test must therefore examine insect survival in both regions.

II. Experiments on the winter survival of insects in bins of grain

At Slough, we tried to determine if infestations of *O. surinamensis* and *S. granarius* could be eliminated using aeration. The insects were placed in wire-gauze cages holding about 0.2kg wheat at the surface of two 4.3m high bins, each holding about 40 tonnes of wheat at 15% m.c. Open-ended tubes, 2m long and 15cm in diameter, were pushed into the centre of the bins, emptied of grain by suction and further cages, attached to strings and containing insects, were inserted into them. The tubes were then refilled with grain. To retrieve the cages, the tubes were emptied of grain and the cages pulled up by their strings. These tubes were, of course, refilled after sampling to prevent air channelling out preferentially. Every month, cages containing 50 adults of each insect species per sampling position were withdrawn and a similar number of controls were set up at sub-breeding temperatures.

The fan of one of the bins was operated manually, and was switched on when the mornings were cool and dry, a procedure used in many grain stores. The second was controlled by a differential thermostat, set to switch on the fan when the difference between ambient and the grain in the bin exceeded 2°C. The centre of the 'differential' bin was usually between 0 and 4°, while that of the other was above 6°C (Table 1).

Table 1. *The hours spent in various low temperature ranges in two aerated bins between October and May*

	°C	above +6	+6 to +2	+4 to +2	+2 to 0	0 to −2	−2 to −6	−4 to −6	below −6
Manual	surface	2523	987	687	353	167	64	6	0
	2m	2762	2025	0	0	0	0	0	0
Differential	surface	2214	1334	966	266	7	0	0	0
	2m	633	946	1960	1248	0	0	0	0
Air temperature		2183	975	770	536	232	81	8	2

S. granarius died before *O. surinamensis* and insects at the bin centres died more quickly than at the surface (Fig. 1.) Insects survived longer in the warmer, manually controlled bin.

The test was repeated in three consecutive years with similar results showing that these two species could be eliminated from the bulk of aerated bins by spring, earlier if a differential thermostat was used. This suggests that quick cooling in grain bins will speed the insects' demise. *O. surinamensis* at the surface of such bins, however, survived. It may prove possible to deal with these by admixing a pesticide with the surface layers, a great saving when compared to turning and admixing the entire bulk.

However, in these tests, the insects were confined in cages and unable to move to more favourable habitats. In other laboratory (Armitage *et al.* 1983) and field tests (Armitage & Stables 1984) we found that although *S. granarius* moved little under most conditions, cooling did cause a wide dispersion of *O. surinamensis*, so they may well accumulate in surface and other peripheral areas. Indeed, where aeration systems suck air through the grain down to the ducts, the aeration fans often

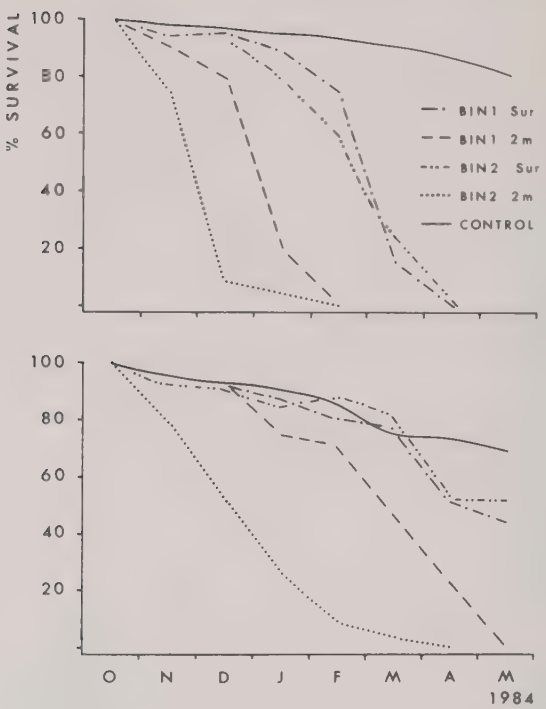

Fig. 1. The survival of S. granarius (above) and O. surinamensis (below) in a bin in which aeration was controlle manually (Bin 1) and another in which it was controlled with a differential thermostat (Bin 2)

discharge large numbers of grain beetles (Armitage & Burrell 1978). The insects only lose thei footing in regions of very high airflow close to the duct (Armitage 1981) so it is unlikely that th movement of insects can be attributed to the force of the airflow.

The effects of changing temperatures on insect behaviour, especially dispersion, need to b further studied. It may prove possible to drive pests into accessible regions of grain stores to facilitat pesticide treatment. However, lower temperatures require increased insecticide concentrations (Ba son 1983). Unfortunately, most studies on insecticide efficacy have been carried out at temperature in excess of 15°C, ignoring the conditions that actually exist in grain stores in the UK, a situatio which our current research programme will hopefully rectify.

VIII. Mite control

Dry grain, at a moisture content in equilibrium with a relative humidity below 70% will only becom suitable for the development of mites when it picks up moisture at the surface in winter. Normall mite numbers in unaerated bins of dry grain will rise steadily after harvest and fall in the spring as th surface grain gains and loses moisture (Armitage 1984). Since the decline in early spring follow winter aeration it has been erroneously attributed to aeration. In fact, the effects of aeration can onl be demonstrated by comparing mite populations in aerated and unaerated bins. By doing this it ha been shown that aeration can delay mite population increase until late spring (Armitage 1980).

In damper grain (above 70% r.h.) mites will occur throughout unaerated bins, but mainly at th surface of aerated bins. Thus it might appear that mites are 'driven' to the surface by aeration. I fact, the migration of mites in response to aeration is unproven and merits further investigation

IX. Future recommendations for grain cooling

Although further research is required to confirm strategies, the following would seem to be desirab when aerating grain in the winter:-

1. Cool to as low a temperature and as quickly as possible (0-5°C by December is normal).
2. Control periods of aeration using a differential thermostat set at 2-4°C (Except with a suctic system, do not use a humidistat to control aeration hours).
3. Whatever the ambient temperature, ensure the grain is aerated for a few hours every month disperse insect metabolic heat.
4. Only aerate grain in equilibrium with an r.h. below 70%; above this it should first be dried.

. Ambient air drying

. The basis for safe drying times

Data for the biological basis for ambient drying techniques are very scarce and the fact is often lamented in papers which model the systems (e.g. Sharp 1982). When drying, our concern is mite and fungal biology. Information on how long the mites take to achieve given numbers under stated conditions of relative humidity and temperature is available (Stratil *et al.* 1980). However, when attempting to select an upper limit for acceptable fungal numbers, one encounters problems quantifying fungi and assessing what comprises a fungal individual.

Ayerst (1969) described fungal growth in terms of the time until germination of conidia and the extension of the germ tube in mm per day. Snow (1949) also studied the time for species to germinate and for the formation of conidia and cleistothecia, but only at one temperature. Both of these studies were carried out on agar. Burrell (1966) and Kreyger (1972) provided more usable data, they defined the time until the formation of 'visible fungi' on grain, without precisely defining the term. Nevertheless, their data provides the only fungal basis for ambient air drying systems.

.I. Mite and fungal populations in ambient air dried grain

To assess the success of the safe drying times currently applied, it seemed logical to examine mite and fungal populations during the drying process using the recommended technique. We therefore examined changes in four 20 tonne bins of barley (Armitage *et al.* 1982), initially at 21% m.c., in equilibrium with a r.h. of 92% (Pixton & Warburton, 1971). Mites were counted after being sieved from 14 samples in each bin. In a homogenous bulk of grain the best assessment of fungal growth during drying is made comparing fungi at 2m, just above the duct where the grain dries almost immediately, with those at 0.5m, just beneath the surface, a position which the drying front reaches last. Fungi were therefore assessed using a dilution plate technique and two media from samples taken at a depth of 0.5m and 2.0m in each bin.

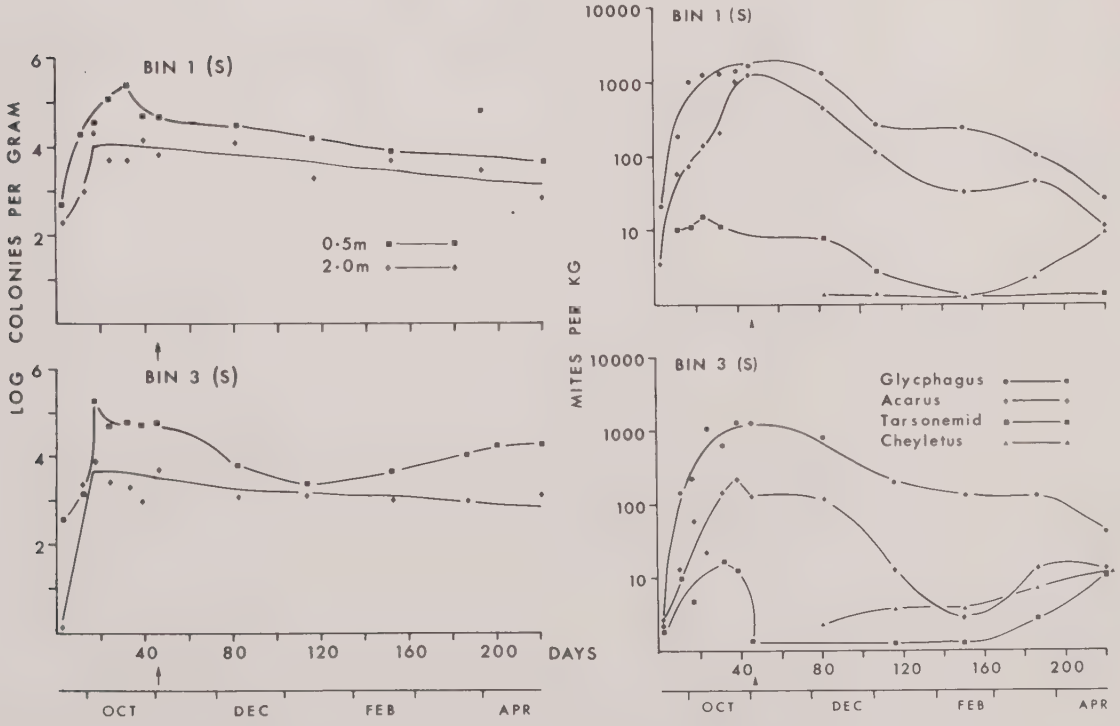

Fig. 2. Changes in storage fungi (left) and mites (right) in two bins of wheat, initially at about 85% r.h. (Arrows indicate the date drying was complete)

Astigmatid mite populations rose sharply during drying, and exceeded 10,000 per kg. Once the drying front had passed through the bulk, however, their numbers collapsed at a rate that was proportional to m.c. Storage fungal numbers also rose sharply during drying but fell only slowly until

the spring. The fungi present were *Pencillium* spp., *Wallemia sebi* and members of the *Aspergillu glaucus* group, while *A. candidus* also occurred at the higher m.c.

Later, the test was repeated using wheat at a moisture content of 19% (85% r.h.). The trend were very similar to those previously described but mite numbers were only just above 1,000 per k and the decline more gradual due to the lower moisture. Fungal numbers were also lower than in th previous experiment but nevertheless exceeded 10,000 colonies per gram. At their peak, mite exceeded acceptable levels, but after drying few were present.

It is worth noting that actual mite numbers were likely to be three times higher than thos described, as sieving only reveals about one-third of all mites present in grain samples (Lynch & Thind 1985). It could be argued that the treatment of peak mite populations is unnecessary becaus they would soon decline. By then, of course, the produce would already have been damaged an tainted. Unfortunately, there is little quantitative information on the damage done by mite popula tions of differing magnitude when sustained for various periods.

XII. Increase of visible fungi and dilution plate counts

In both the previous tests, numbers of storage fungi exceeded 100,000 colonies per gram, a seemingl high number, but not easy to relate to Burrell's and Kregyer's criterion, 'visible mould'. Therefore, laboratory test was carried out in which weekly dilution plate counts and visible fungal assessment were made of triplicate samples of aerated and unaerated wheat prepared to m.c.'s in equilibriun with 85% and 92% r.h. The temperature chosen was 17°C, about the maximum occurring durin ambient drying. To make a visible assessment, about 200 grains were examined under a low powe binocular microscope and the number 'visibly moulded' noted.

The earliest sign of fungi in this test was the appearance of mycelium in the crease of the grain but the first conspicuous sign was the appearance of conidia, followed by cleistothecia of *Eurotiun* spp., which occurred only on unaerated grain at 85% r.h. Differences between aerated and unaerate grain were probably unimportant and attributable to slight fluctuations in the r.h. control. Dilutio plate counts reached higher levels on the damper grain (Table 2). By one week, counts exceede 10^5/g at 92% r.h. but this level was reached only after 3 weeks at 85% r.h. At 92% r.h. the mos important fungi were *Penicillium* spp. and *A. versicolor*, while at 85% r.h. *A. glaucus*, *Penicillium* spp. and *Wallemia sebi* all occurred in similar numbers. The first appearance of conidia appeared t coincide with a dilution plate count of 10^5 colonies/g (Fig. 3) and the correlation of visible fungi wit dilution plate count seemed to vary with r.h. (Table 2). Thus the criterion of 'visible fungus' does no seem to be sufficiently reliable as a basis for recommended drying times for ambient air dryin systems.

Table 2. *The increase of storage fungi (log colonies/g) in aerated (a) and unaerated (u) wheat*

Week		0	1	2	3	4	5	6	7	9	11
92% r.h.	a	1.6	5.5	6.6	7.3	7.6					
	u	1.6	5.8	6.7	7.3	7.6					
85% r.h.	a	0	2.8	3.5	4.3	–	5.6	6.3	5.9	5.6	5.7
	u	0	2.7	3.1	5.4	–	6.1	6.3	6.7	6.4	6.2

Different grains have different susceptibilites to fungal attack, for instance, barley is said to b more resistant than wheat. Rapeseed becomes bound together with mycelium before conidial head become visible (Burrell *et al.* 1980) and this may restrict airflow, retard drying and therefore permi infestations. Drying recommendations based on one variety of grain can therefore scarcely b adequate.

XIII. Fungal mite interactions

A further complication, that so far has been ignored in models of driers and in experiments on funga growth on grain, is the interaction between mites and fungi. Some fungi are eaten by mites (Griffith *et al.* 1959), others are toxic to them (Solomon *et al.* 1964) and recent work has shown that mit infested grain contains fewer fungi than non-infested grain (Armitage & George 1986). I practice, of course, the organisms occur together, while in most experiments on fungal growth, mite are either deliberately excluded or ignored, so their effects are negligible or unknown.

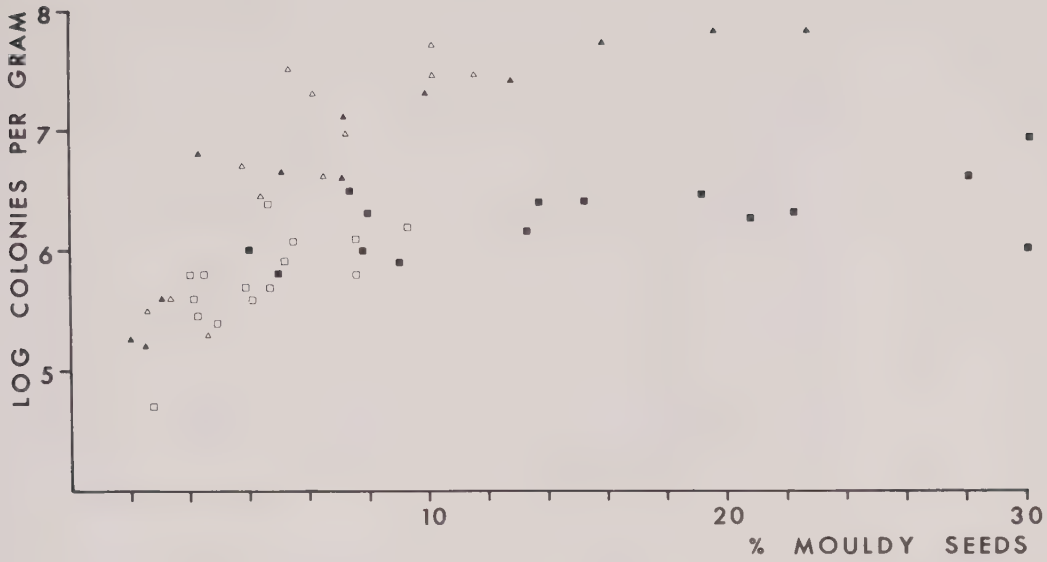

Fig. 3. The correlation of dilution plate count with visible mould at 92% r.h. (triangles) and 85% r.h. (squares) in aerated (open symbols and unaerated (closed symbols) wheat.

eferences

rmitage, D.M. (1980) The effect of aeration on the development of mite populations in rapeseed. *Journal of Stored Products Research* **16**, 93-102.

rmitage, D.M. (1981) Effect of high air velocities on adhesion to and behaviour on a perforated surface of arthropod pests of grain. *Entomologist's Monthly Magazine* **114**, 59-61.

rmitage D.M. (1984) The vertical distribution of mites in bulks of stored produce. In *Acarology* **2**, (D.A. Griffiths, C.E. Bowman, eds), 1006-1013. Chichester; Ellis Horwood.

rmitage D.M.; Burrell, N.J. (1978) The use of aeration spears for cooling infested grain. *Journal of Stored Products Research* **14**, 223-226.

rmitage, D.M.; Stables, L.M. (1984) Effects of aeration on established insect infestations in bins of wheat. *Protection Ecology* **6**, 63-73.

rmitage, D.M.; George, C.L. (1986). The effect of three species of mites upon fungal growth in wheat. *Applied Acarology* (in press).

rmitage, D.M.; Llewellin, B.E.; Burrell, N.J. (1982) Changes in fungal and mite populations in grain during prolonged drying and subsequent storage. In *Proceedings, Third International Drying Symposium* (J.C. Ashworth, ed.), 193-201. Wolverhampton; Drying Research Ltd.

rmitage, D.M.; Day, P.G.; Lewis, G.J. (1983) The effect of aeration on the vertical distribution of grain beetles (Coleoptera) in wheat. *Bulletin of Entomological Research* **73**, 233-237.

yerst, G. (1969) The effects of moisture and temperature on growth and spore germination in some fungi. *Journal of Stored Products Research* **5**, 127-141.

arson, G. (1983) The effects of temperature and humidity on the toxicity of three organophosphorus insecticides to adult *Oryzaephilus surinamensis* (L.). *Pesticide Science* **14**, 146-152.

urges, H.D.; Burrell, N.J. (1964) Cooling bulk grain in the British climate to control storage insects and to improve quality. *Journal of the Science of Food and Agriculture* **1**, 32-50.

urrell, N.J. (1966) Refrigerated damp grain storage. *Pest Infestation Research 1965*, 17-19. London; H.M.S.O.

urrell, N.J.; Knight, G.P.; Armitage, D.M.; Hill, S.T. (1980) Determination of the time available for drying rapeseed before the appearance of surface moulds. *Journal of Stored Products Research* **16**, 115-118.

otton, R.T. (1956) Prevention and control of insect infestation in farm stored grain. In *Pests of stored grain products*, Chapter IV. Minneapolis; Burgess.

avid, M.H.; Mills, R.B.; White, C.D. (1977) Effects of low temperature acclimation on developmental stages of stored product insects. *Environmental Entomology* **6**, 181-184.

astham, L.E.S. Segrove, F. (1947) The influence of temperature and humidity on instar length in *Calandra* (*Sitophilus*) *granaria* Linn. *Journal of Experimental Biology* **24**, 79-94.

vans, D.E. (1981) Thermal acclimation in several species of stored grain beetles. *Australian Journal of Ecology* **5**, 371-378.

Granovsky, T.A.; Mills, R.B. (1982) Feeding and mortality of *Sitophilus granarius* (L.) adults during simulated winter farm bin temperatures *Environmental Entomology* **11**, 324-326.

Griffiths, D.A.; Hodson, A.C.; Christensen, C.M. (1959) Grain storage fungi associated with mites. *Journal of Economic Entomology* **52**, 514-518

Holman, L.E. (1960) *Aeration of grain in commercial storages.* Marketing Research Report 178. Washington; US Department of Marketing Services Research Division.

Howe, R.W. (1965) A summary of estimates of optimal and minimal conditions for population increase of some stored products insects. *Journal of Stored Products Research* **1**, 177-184.

Hyde, M.B.; Burrell, N.J. (1969) Control of infestation in stored grain by airtight storage and cooling. *Proceedings, Fifth Insecticide and Fungicide Conference,* 412-419.

Hyde, M.B.; Burrell, N.J. (1973) Some recent aspects of grain storage technology. In *Grain storage : part of a system.* (R.N. Sinha; W.E. Muir, eds). 313-341. Westport; Avi Publishing.

Kreyger, J. (1972) *Drying and storing grains, seeds and pulse in temperate climates.* Wageningen; Institut Voor Bewaring en Verweking van Landbouw Produkten.

Lynch, S.M.T.; Thind, B.B. (1985) A comparison of sieving and oil flotation methods for quantitative assessment of mite populations in grain. *Journal of Stored Products Research* **21**, 35-40.

Mathlein, R. (1961) Studies on some major storage pests in Sweden with special reference to their cold resistance. *Statens Växtskyddanstal Meddelanden* **12:83**, 49pp.

Obretenchev, P. (1983) Action of low and high lethal temperatures on various stages of the saw toothed grain beetle. *Oryzaephilus surinamensis* L Coleoptera, Cucujidae. *Rastenievadni Nouki (Sofia)* **20**, 45-50.

Pixton, S.W.; Warburton, S. (1971) Moisture content/relative humidity equilibrium of some cereal grains at different temperatures. *Journal of Stored Products Research* **6**, 283-293.

Robinson, W. (1928) Determination of the natural undercooling and freezing points in insects. *Journal of Agricultural Research* **37**, 749-755.

Sharp, J.P. (1982) A review of low temperature drying simulation models. *Journal of Agricultural Engineering Research* **27**, 169-190.

Snow, D. (1949) The germination of mould spores at controlled humidities. *Annals of Applied Biology* **36**, 1-13.

Solomon, M.E.; Hill, S.T.; Cunnington, A.M.; Ayerst, G. (1964) Storage fungi antagonistic to the flour mite (*Acarus siro* L.). *Journal of Applied Ecology* **1**, 119-125.

Stratil, H.V.; Stratil, H.H.; Knülle, W. (1980) Untersuchungen über die spezifische Vermehrungsrate von Populationen der in Lagergetreide lebenden Milbe *Glycyphagus destructor* (Schrank 1781) bei verschiedenen Temperatur- und Luftfeuchtebedingungen. *Zeitschrift für angewandte Entomologie* **90, 209-220.**

Ushatinskaya, R.S. (1948) The importance of sudden and gradual falls of temperature on the 'cold stability' of the granary weevil (*Calandra granaria* L.). *Zoologicheskii Zhurnal* **27**, 495-502.

Ushatinskaya, R.S. (1950) The influence of the humidity of the surroundings and of food on the cold resistance of the granary weevil *Calandra granaria. Zoologicheskii Zhurnal* **29**, 341-349.

ɔoilage and Mycotoxins of Cereals and other Stored Products.
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New Developments in Pest control

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Summary

Current developments in pest control reflect the problems created by recent changes in the cereal industry, notably the increased reliance on admixture of contact insecticides and fumigation. Improved application techniques and formulations, and an understanding of the movement of pesticide in grain bulks and the role of the vapour phase, are all seen as important keys to improving control while reducing harmful residues in food.

Safer alternatives to existing liquid fumigants are discussed, as is the manipulation of the gaseous atmospheres of well-sealed stores.

Insect juvenile hormones and anti-hormones offer good prospects for the future long-term storage of cereals, without accruing toxic residues.

I. Introduction

Use of the term "new" at a scientific meeting is always daunting, for in truth there is very little in pest control that is novel. We make much today of our work with insect hormones and growth regulators, but we can read in Cato's *De Re Rustica* (234-149 BC) that olive oil-lees "caused the caterpillars to swell and to burst". What can this mean, except that he had observed the juvenile hormone mimic effects of terpenes, thus anticipating the work of Wigglesworth, Karlson, Williams and Bowers by a mere two millennia?

Mr Wilkin's paper has indicated the changes that have taken place in the UK cereal industry during the past 10 years, and the consequential change in attitudes to pest control. Admixture of pesticides is now seen by hard-headed businessmen in the grain trade as insurance against downgrading due to insect or mite spoilage, with fumigation held as a last resort for rapid disinfestation. Used in accordance with MAFF advice these practices should cause few problems. Nevertheless we have to be aware of problems that might occur, such as inadequate or over-dosing; retreatment of grain changing hands in commerce, raising the possibility of cumulative or multiple residues; and residues that survive food processing (Wilkin & Fishwick 1981). However, reports of the Working Party on Pesticide Residues for 1977-81 and 1982-5 (MAFF 1982, 1986) have been reassuring in that pesticide levels found in both home-grown and imported wheat have been generally low, and within internationally accepted tolerances. At the same time, resistant strains of insects and mites are becoming widespread, and multiple- or cross-resistance threatens our limited range of permitted pesticides. There are international pressures to withdraw the current liquid fumigants on health

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grounds. Due to economic pressures and more stringent evaluation, the rate of commercial develop ment of new pesticides has slowed dramatically. Thus there are no immediate candidates to augmer our dwindling armoury of approved pesticides.

These are the problems, and most of the "new" developments are concerned with solving ther and in reducing the use of toxic pesticides to an effective minimum.

III. Pesticide application and dose achieved

Contact insecticides are applied to grain or to the fabric of stores as approved or registered formula tions: emulsions (sprays), dusts or wettable powders. In devising strategies to reduce pesticide usag our first reliance is on the rapid and timely detection of cryptic infestation and the consequen decision on when, what, and where to treat. These aspects are dealt with in Mr Pinniger's paper. It i then important to achieve the recommended dose.

Crucial among our lack of data is information on the doses actually achieved on grain or o surfaces by farmers and storemen aiming for a particular recommended level. Poor stability o formulation, inadequate mixing of diluted emulsion during spraying, uneven flow-rate of grain o pesticide during admixture, losses during conveying after treatment and spray or dust drift - all thes factors may contribute to uneven and inaccurate dosing of cereals. We have found pesticide (dust) o grain in untreated bins close by those being treated, and we have found pesticide deposits in th cobwebs and debris of roof spaces and corners of granaries. Once again,however, the Food Surveil lance reports (MAFF 1982, 1986) suggest that, in general, there are no problems from over-dosing

Improvements in both equipment and handling would enable pesticides to be used more effec tively, allowing lower dosage rates and reducing the ultimate residues in food products. The possibil ity of developing such improvements is being explored jointly by the Slough Laboratory and th National Institute of Agricultural Engineering.

How much of the pesticide actually gets on to the grain or fabric, and how evenly, depends on th method of application, the flow-rate and the depth of the grain stream being treated. Some years ag now, by analysing individual grains in 25 g samples removed from the flow into bins after spraying we were surprised to find that all 550-odd grains in a given sample had received some pesticide though the individual concentrations ranged from 0.1 mg/kg to 424 mg/kg (Rowlands 1975) Similar results can be demonstrated under different practical conditions. Even if all grains are no dosed directly or if dosing is extremely erratic - as perhaps when mixing of spray is poor, or the dus applicator is blocking - the consequences are not necessarily bad for pest control, as has sometime been implied. Indeed, a dose concentrated in just a few highly-toxic grains of a bulk may initiall prove more effective in bioassay than an evenly-distributed dose (Rowlands 1975), and large-scal treatments have been undertaken deliberately to exploit this "uneven dose" strategy and to define it potential (Minett & Williams 1976). Any advantage may well betransient, since the pesticide wil usually redistribute itself throughout the grains and intergranular spaces of the bulk during a mont or so of storage. This premise was based initially on a number of laboratory experiments of differin scale up to kilo weight. Recent data from our colleagues Fishwick and Wilkin (personal communica tion) have shown it to be true of the tonne scale also.

There is much to be gained from better understanding the movement of pesticide in grain bulk and exploiting any control (and residue) advantages inherent in degrees of uneven dosing. This i especially so if the formulation used can be tailored to enable easy removal of any pesticide left at th end of the storage period, as Desmarchelier (1985) has demonstrated by using dust formulations wit a high partition coefficient relative to that of the grain. This ties in aptly with the early detection an diagnosis of the problem as mentioned above. Depending on where the infesting species attack th grain or where they breed, whether they are primarily surface, germ or endosperm feeders, it shoul be possible by manipulative formulation to deliver the pesticide in the right dose, in the right plac and at the right time, so minimising residues and any need for re-treatment.

IV. The vapour phase of contact insecticides

In considering the movement of pesticides through a grain bulk there are two interrelated aspects o contact insecticide vapour to be noted. One is obviously the contribution that the vapour phas (volatility) makes to translocation through a grain bulk. We have demonstrated a surprising amoun of vapour-phase transfer of malathion (not usually reckoned among the more volatile contac pesticides!) between wheat grains in laboratory studies at ambient temperature (Rowlands & Bram hall 1977) and have established that the amount absorbed is not related to the size, weight o moisture content of the grains. Nor is such transfer associated with water exchange between grains though it is linked with seed viability.

The second aspect is the high intrinsic toxicity of the vapour itself, and its fumigant-like rapi entry through the insect spiracles, as distinct from the slower penetration through the cuticle. Fo example, malathion at its equilibrium concentration 60 μg/litre (i.e. *ct* product approx. 0.

mgh/litre) killed *T. castaneum* pupae and at 500 μg/litre killed the mite *Acarus siro*. To what extent does the rapid entry of vapour from a pesticide deposit contribute to any knock-down effect? From the insect's standpoint on any treated surface (be it grain or fabric) the vapour layer perhaps 0.1 to 0.5 mm above that surface, depending on posture and length of legs (i.e. *Cryptolestes* or *Sitophilus*), may be more lethal than the amount it picks up by contact or ingestion.

On the debit side, we would expect that vapour losses would contribute to the difference mentioned earlier between intended and achieved doses, and also to the initial loss of the achieved dose, particularly from the surface of the bulk. Thus we might expect that forced aeration would enhance vapour loss of contact insecticides. Surprisingly, however, Thomas (1984) showed that in comparing 20-tonne bulks of unaerated wheat treated at 4 mg/kg with chlorpyrifos-methyl (known to be quite volatile) and similarly treated bulks with upward aeration at 10 m^3/ton/h there were no differences in insect bioassays on, or pesticide residues in, samples removed from those bulks during storage for 16 weeks.

The whole subject of "pesticide vapour" abounds with folklore and anecdotal information; only a few workers (Storey 1972, Rowlands & Bramhall 1977, Desmarchelier *et al.* 1977, Rowlands 1985) have so for attempted scientific studies. Desmarchelier *et al.* (1977) defined the factors involved in the lethal action of contact insecticide vapour as: vapour toxicity, vapour pressure, degree of absorption on the grain and the rate at which equilibrium absorption is obtained. A proper study of these parameters would seem to offer considerable potential for pest control at much reduced pesticide dosages.

. Alternative liquid fumigants

Fumigation is used for the rapid disinfestation of commodities *in situ* and is often undertaken as an emergency measure, when, for example, grain becomes infested which must either be sold immediately or kept for long-term storage. It is unfortunate that poor storage conditions (the usual cause of infestation) also conspire to make satisfactory fumigation more difficult.

In the majority of stores in the U.K., the only fumigants used today are the liquid fumigants carbon tetrachloride and ethylene dichloride, and solid aluminium phosphide (phosphine). Methyl bromide can be used for bagged grain in stacks under gas-proof sheets but is not suitable for grain unless some means of air circulation to distribute the gas through the bulk is available, as in purpose-built deep silos at ports and transit areas.

Crucial factors in the success of a treatment are the air-tightness of the store, chamber or stack, and the maintenance of the right concentration of gas for the period necessary to deal with the pest concerned. Fumigation confers no protectant effect; once disinfested, the bulk is liable to reinfestation if the primary source has not been dealt with. The toxicity of a fumigant to an insect or mite is influenced by both the gas concentration (c) and the time (t) for which that concentration is maintained, and in designing fumigation techniques it is necessary to know the product (ct) required for successful control in practice. Extensive studies on the toxicity of fumigants to insects and mites under varying conditions have been carried out at the Slough Laboratory and the data accumulated have been used to work out dosage schedules for commercial use.

The main appeal of liquid fumigants is ease of application. Many of us remember use by farmers of watering cans to pour carbon tetrachloride on top of stacked grain. But, in 1980, the Pesticide Safety and Precautions Scheme (PSPS) deemed it wise to restrict application of liquid fumigants to Professional Operatives who should have adequate means of personal protection.

Carbon tetrachloride (CTC) is not particularly toxic to insects. In liquid fumigant mixtures it chiefly acts as a carrier for the other component which tends to be sorbed and be slow in moving down a column of grain in a deep bin. Ethylene dibromide was formerly used in mixture with CTC and ethylene dichloride but has since been withdrawn. It tends to remain near the surface and in its absence any increase of the proportion of ethylene dichloride (EDC) in the current 1:1 mixture with CTC is not able to compensate for some loss of efficacy in this zone. Hence the mixture, although extensively used, is prone to be a marginal control procedure.

It has been known for many years that CTC is a chronic poison, causing lesions of the liver and other organs. Cases have occurred of deaths among cattle fed treated grain which had been insufficiently aired. Recently, the Health and Safety Executive (1981) have listed CTC as a "substance likely to have carcinogenic potential in man" and have reduced the Threshold Limit Value (TLV) from 10 to 5 ppm.

Work has been going on at Slough since 1973 to find a safer replacement for CTC. Methyl chloroform (1,1,1-trichloroethane, MC) has very similar properties to CTC and will penetrate to the bottom of deep silo bins without difficulty. Sorption characteristics and residue data are similar for both compounds.

It is also very much safer to man (TLV 350 ppm) and after extensive testing in the U.S.A. there was no evidence of carcinogenicity (WHO, 1979). Unfortunately, it is only of the same low toxicity to insects as CTC, such that control with MC alone, even at high temperatures, is unlikely, requiring a ct product at 25°C of more than 20,000 mgh/litre to be achieved in all parts of the grain bulk. The

chief problem is that MC tends to accumulate at the bottom of bins after a surface applicatio resulting in low dosages in the surface layers. However, again like CTC, it can act as a good *carri* for other compounds, and our research has reached the point where MC can be notified as replacement for CTC (Bell & Rowlands 1983).

Work on a replacement for EDC to be mixed with MC as a grain fumigant mixture has been progress at Slough for some time. Bearing in mind the long time-scale involved in approval of ne compounds, we turned to one which was already widely used on foodstuffs, namely methyl bromic (MB), but which was unable to penetrate deep grain bulks without assistance. Much success has bee obtained in both laboratory and field trials in silos, using a 5% or 10% solution of methyl bromide methyl chloroform, at the rate of 1.0 and 0.5 litre/tonne grain (Bell & Rowlands 1983). Mo recently a 20% mixture has been evaluated with success on floor-stored grain. This mixture may al be suitable for silo treatments.

Laboratory toxicity tests against insects of MB/MC mixtures at various concentrations in th 1:20 ratio show no enhancement of MB toxicity due to the presence of MC. This suggests that fie trials can be assessed in terms of the *ct* products obtained for MB only, and the contribution of MC the control obtained can be ignored unless the level of MC happened to be *very high*. The mai advantage of using the mixture is likely to be the better distribution of MB in bulk commoditi which has been observed in field trials.

VI. Controlled atmospheres

The spread of resistance to pesticides in Australia has led to a vigorous interest there in efficie sealing of structures, both for conventional fumigation, and for a re-assessment of the ancie technique of controlled atmospheres. A similar interest has been evident in the USA where, lik Australia, centralised storage on a large scale is normal practice.

Just as adequate sealing of the grain bulk or store is essential for successful fumigation, it is als *a priori* for the techniques of controlled atmosphere storage. Leakage impairs the lethality of th atmosphere and maintaining the concentration of carbon dioxide or burner gas puts up the cost of th exercise.

The techniques involve control of infestation by manipulation of the gas composition of the sto or bulk environment to a state of oxygen deficiency or carbon-dioxide richness. There are sever ways of achieving this: by natural reduction of oxygen during airtight storage, reduction of oxygen b exogenous micro-organisms (e.g. *Aspergillus* spp.), or by purging the grain stack/silo with (a) bul carbon dioxide, (b) nitrogen, or (c) combustion gases from a portable generator. The drawbacks the first two should not need emphasising to the readers of this paper. Purging with nitrogen is ver expensive since virtually the entire atmosphere has to be replaced and maintained to be effectiv

Work at Slough on the lethal aspects of various atmospheric gas mixtures incorporating carbo dioxide has so far concentrated on the gas ratios produced by purging with bulk carbon dioxide catalytic converters and thermal generators, i.e. low-oxygen exhaust gas. Our colleagues Bell an Wilkin took the opportunity last year to participate with the USDA in field trials on controlle atmosphere storage using burner gas generated by an exothermic burner. Their preliminary asses ment suggests that the on-site burner gas generator, despite its initial high cost, provides a cheap purging gas than bulk carbon dioxide. In either case, the operating costs compare favourably wit current techniques involving contact insecticides or fumigants. In the immediate future, our researc aims are to find the most effective mixtures (especially the carbon dioxide level required in burn gas), using bulk carbon dioxide or combusted atmosphere (liquid propane or natural gas), against th full range of pest species, including mites; and to establish purging times and bleed rates fo maintaining atmospheres in various types of structure, defining innate leakage rates by pressu testing. It is also necessary to find out how much carbon dioxide is removed from combuste atmospheres applied to grain of different moisture contents.

In different and less conventional approaches, investigators elsewhere are looking at atm spheres containing up to 1500 ppm of ozone, and also the use of beta- or gamma-radiation microwaves under vacuum as disinfestation techniques.

VII. Novel compounds

Perhaps the most exciting potential for pest control is offered by the insect growth regulators (IGRs a group which includes disruptors of cuticle formation as well as compounds that affect the endocrin systems. It is the latter that we shall consider here, particularly the insect hormone analogues an anti-hormones. They have striking advantages over conventional pesticides in being virtually no toxic to mammals and demonstrating high degrees of biological activity and specificity (Edwar 1983).

Briefly, the hormones act by interfering with insect development at crucial stages: in the case the synthetic juvenile hormones they are applied at the final larval stage, producing developmenta

abnormalities that culminate in a pupal-adult intermediate destined to die at pupation or emergence. The timing and dosing can be critical, however, since the natural function of the hormone is to maintain the larval state. Overdosing in the early larval stages can produce extra moults and in some cases "permanent" or outsize larvae,i.e. feeders ....not a desirable situation for grain storage.

Since identification of the chemical structures of the insect juvenile hormones, a number of analogues (mimics) have been synthesised and, in fact, commercial juvenile hormone analogues (JHAs) have been available for some 13 years now. Despite this, they have only slowly come into practical use for pest control, despite some initial success in the public health sphere (Edwards 1981). Initial problems with stability and formulation, high cost and varying degrees of effectiveness, especially against stored product insects (notably a poor performance against *Sitophilus* spp.), all hindered the advancement of JHAs as grain protectants.

For example, the naturally occurring JH-l and the commercial analogue, methoprene, are very effective against most stored product pests except *Sitophilus* spp. Moreover, Dyte (1972) showed that a strain of *Tribolium castaneum* resistant to a wide range of conventional pesticides was also less sensitive to JH-l. This has highlighted the importance of testing the effectiveness of any new compound, whatever its mode of action, against insecticide-resistant strains.

Recently, however, a number of new compounds with hormonomimetic effects on insects have been discovered. One of these, fenoxycarb or ethyl N-2-(4-phenoxyphenoxy) ethyl carbamate, has shown potential as a grain protectant and exhibited high JH-activity against both susceptible and insecticide-resistant strains of *Sitophilus* spp. (Edwards & Short 1984), as well as a wide range of susceptible and resistant strains of other storage insects. Once again some degree of tolerance to fenoxycarb was found in the multiple-resistant strain of *T. castaneum* (Thind & Edwards 1986), however.

In laboratory tests fenoxycarb proved fully effective against a range of storage pests at 5 mg/kg and there was no loss of effectiveness against *Sitophilus* spp., even after treated grain was stored at 25°C and 70% r.h. for 12 months. Together with its low mammalian toxicity (16,500 mg/kg rat, acute oral LD_{50}) this indicates considerable potential as a safe grain protectant, and we currently have one tonne-scale trials in progress to measure its performance during two years of practical storage.

However, there remain some obvious practical drawbacks with JHAs. Generally they do not kill adults and their lethal effect is delayed, making control a prolonged business with no immediately observable effects. Moreover, JHAs exert their effect at the late larval stage, by which time any damage to the grain has been done! So, in recent years interest has focussed on the so-called "anti-hormones" which, if they can prevent the synthesis, secretion or reception of the natural juvenile hormones, might disrupt the development of insects in the early larval stage, and also affect such hormone-regulated processes as diapause and reproduction.

A number of compounds with varying degrees of anti-hormone effects have been isolated from a variety of sources (Edwards 1983), though some, such as the precocenes, have little pest control potential. The compound fluoromevalonate, which inhibits biosynthesis of juvenile hormone, produces premature metamorphosis (precocious pupation) in Lepidoptera and is a useful reference standard for comparison of anti-JH effects.

At Slough we have studied another inhibitor of juvenile hormone biosynthesis: a natural product of certain fungi such as *Penicillium brevicompactum*. This compound, compactin, is the most potent *in vitro* inhibitor of juvenile hormone biosynthesis yet discovered (Edwards & Price 1983). There is a snag, however. Unlike fluoromevalonate, compactin does not show anti-juvenile hormone activity when applied to insects *in vivo*. The reasons for this are still being investigated.

Such studies have indicated that disruption of the endocrine system of insect larval stages is possible in a number of ways that provide a basis for developing potent, yet safe, new pesticides.

III. Conclusions

Current new developments in pest control reflect the problems created by recent changes in the cereal industry, notably the increased reliance on admixture with contact insecticides and fumigation. Improved application techniques, purpose-designed formulations, and understanding of translocation of pesticide in grain bulks and the role of the vapour, all offer prospects of improving control while reducing harmful residues in food. Manipulation of the gaseous atmospheres of well-sealed bulks or stores is also of potential. Insect juvenile hormones and antihormones provide a good prospect for future prolonged storage of cereals without accruing toxic residues.

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References

Bell, C.H.; Rowlands, D.G. (1983) The future for liquid fumigants. *Proceedings of the Sixth Pest Control Conference, Cambridge, 1983*, Session 7, Paper 19, 10pp.

Cato, M.V. (234-149 BC) *De Re Rustica* (W.D. Hooper & H.B. Ash, Transl. 1967). London; Loeb Classical Library, William Heinemann.

Desmarchelier, J.M. (1985) Behaviour of pesticide residues. *Proceedings of the International Symposium on Pesticides and Humid Tropical Grain Storage Systems, ACIAR, Manila, 1985* (in press).

Desmarchelier, J.M.; Banks, H.J.; Williams, P.; Minett, W. (1977) Toxicity of dichlorvos vapour to insects in aerated and non-aerated wheat and comparison of the vapour action of dichlorvos and malathion. *Journal of Stored Products Research* **13**, 1-12.

Dyte, C.E. (1972) Resistance to synthetic juvenile hormones in a strain of the flour beetle, *Tribolium castaneum*. *Nature, London* **238**, 48-9.

Edwards, J.P. (1981) The biology, importance and control of pharaoh's ant (*Monomorium pharaonis* L.) infestations in hospitals. *Journal of Infection Control Nursing (Nursing Times)* **15**, 2-4.

Edwards, J.P. (1983) Novel methods of pest control. *Proceedings of the Sixth Pest Control Conference, Cambridge, 1983*, Session 3, Paper 6, 8pp.

Edwards, J.P.; Price, N.R. (1983) Inhibition of juvenile hormone III biosynthesis in *Periplaneta americana* with the fungal metabolite compactin (ML-236B). *Insect Biochemistry* **13**, 185-189.

Edwards, J.P.; Short, J.E. (1984) Evaluation of three compounds with insect juvenile hormone activity as grain protectants against insecticide-susceptible and resistant strains of *Sitophilus* species (Coleoptera, Curculionidae). *Journal of Stored Products Research* **20**, 11-15.

Health and Safety Executive (1981) *Threshold Limit Values, 1980*. Guidance Note EH 15/80, London; HMSO.

M.A.F.F. (1982) *Report of the Working Party on Pesticide Residues (1977-81)*. Food Surveillance Paper No. 9. London; HMSO.

M.A.F.F. (1986) *Report of the Working Party on Pesticide Residues (1982-85)*. Food Surveillance Paper No. 16. London; HMSO.

Minett, W.; Williams, P. (1976) Assessment of non-uniform malathion distribution for insect control in a commercial wheat silo. *Journal of Stored Products Research*, **12**, 27-33.

Rowlands, D.G, (1975) The metabolism of contact insecticides in stored grain. III. *Residue Reviews*, **58**, 113-155.

Rowlands, D.G. (1985) Background studies on the metabolism of residual insecticides in grain. *Proceedings of the International Symposium on Pesticides and Humid Tropical Grain Storage Systems, ACIAR, Manila* (in press).

Rowlands, D.G.; Bramhall, J.S. (1977) The uptake and translocation of malathion by the stored wheat grain. *Journal of Stored Products Research* **13**, 13-20.

Storey, C.L. (1972) The effects of air movement on the biological effectiveness and persistence of malathion in stored wheat. *Proceedings of the North Central Branch of the Entomological Society of America* **27**, 57-62.

Thind, B.B.; Edwards, J.P. (1986) Laboratory evaluation of the juvenile hormone analogue fenoxycarb against some insecticide-susceptible and resistant stored product beetles. *Journal of Stored Products Research* (in press).

Thomas, K.P. (1984) The effects of low-volume aeration on residues of chlorpyrifos-methyl in a grain bulk. *MAFF R&D Report : Storage Pests 1983*, 1-3. London; HMSO.

W.H.O. (1979) *IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans*, Vol. 20. *Some halogenated hydrocarbons*. Lyon; IARC.

Wilkin, D.R.; Fishwick, F.B. (1981) Residues of organophosphorous pesticides in wholemeal flour and bread produced from treated wheat. *Proceedings, 1981 British Crop Protection Conference, Pests & Diseases, Brighton* **2**, 183-187.

Inspired by Cato (234–149 BC)

Spoilage and Mycotoxins of Cereals and other Stored Products.
Edited by B. Flannigan

MICROORGANISMS IN CEREALS AND OTHER STORED PRODUCTS

Factors Affecting Fungal Colonization of Grain

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I. Summary

Fungal colonization of grain begins soon after the ear emerges from the flag leaf sheath. *Cladosporium* usually predominate but numbers of all species vary with climate, nutritional status of the ripening grain and extent of lodging. Storage species are usually few before harvest but *Aspergillus flavus* may be important in warm climates. Further inoculum of storage species is added during harvesting, drying and storing. Moulding in store depends on available water, temperature and intergranular gas composition but may be modified by other factors. Fungal growth is prevented at water activity levels (a_w), less than about 0.65 a_w. With increasing a_w, microbial activity increases and may cause heating, allowing thermophilic species to develop. Carbon dioxide may inhibit moulding in the deeper grain. *Aspergillus* species are often good indicators of storage conditions.

II. Introduction

Fungi colonizing grain have been classified into two groups, *field* and *storage* fungi. Field fungi colonize the grain before harvest and rarely develop further in store, while storage fungi are few before harvest but increase subsequently. However, studies of grain colonization in warmer climates suggest that such a rigid division may not be tenable. Even in temperate climates, an intermediate group has been proposed for fungi that sometimes grow in store. In both field and store, fungal growth is determined by the water, temperature and nutritional requirements of the fungus and its responses to fluctuations in them, to gas composition and to competition with other microorganisms. If interactions between these factors and colonizing fungi are understood, the conditions leading to a particular microflora can be determined.

III. Fungal development before harvest

Newly emerged ears of cereal crops are soon colonized by microorganisms (Hill & Lacey 1983a). Bacteria develop first but are soon followed by yeasts and filamentous fungi (Table 1). Inoculum comes from the air and is splashed from the soil or from other plants. Nutrition is provided by solutes from microbes, pollutants and plant cells and changes quantitatively and qualitatively as the grain ripens. Environmental conditions around the developing grain fluctuate widely between day and night, with extremes of temperature, humidity and radiation. During ripening, the water content of

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the tissue declines and atmospheric humidity and rainfall become more important in determining water availability.

Table 1. *Predominant field fungi on cereal grains*

Yeasts and yeast-like fungi	Filamentous fungi
Cryptococcus spp.	*Alternaria alternata*
Rhodotorula glutinans	*Botrytis cinerea*
Sporobolomyces roseus	*Cladosporium* spp.
Trichosporon cutaneum	*Epicoccum purpurascens*
Torulopsis ingeniosa	*Fusarium* spp.
Aureobasidium pullulans	*Phoma* spp.

Fungi are variously affected by the environment. Damp weather usually favours fungal growth and increases populations of many species. For instance, *F. graminearum* causes most wheat ear blight in Canada when surfaces at anthesis are wet for 48-60 h and temperatures are 20-30°C. Most maize is infected when >70-80 mm rain falls on 6-9 days in August (Sutton 1982). In the Transvaal, South Africa, *F. moniliforme* on maize is favoured by the sub-tropical climate of the north, *F. subglutinans* by the wetter climate of the east and *F. graminearum* by the intermediate climate of the west (Marasas *et al.* 1979). Overwintering of grain in the field can also lead to severe *Fusarium* infection as with *F. sporotrichioides* on millet in the Ukraine and with other species on maize in Canada (Joffe 1965, Mills & Frydman 1980). Also, lodging can slow drying and favour some storage penicillia.

Susceptibility of the host plant to fungal infection is largely determined by its genetic constitution but can be altered by changes in the environment. For instance, drought stress and high temperatures at the late milk to dough stage can increase infection of both resistant and susceptible maize cultivars to *A. flavus*, with more when the mean temperature is near 27°C than when it is below 26°C (Hill *et al.* 1985). However, resistance is often assessed by inoculating mature kernels, ignoring other important infection routes, e.g. the silks. Most *A. flavus* infection occurs within 2-3 weeks of silk emergence. Resistance to insects can also decrease infection by decreasing the number of sites of feeding damage through which fungi can penetrate.

IV. Inoculum of storage fungi

Storage fungi are seldom detected before harvest, yet even small quantities of grain will mould in a way that can be predicted from their water activity (a_w). Undoubtedly some of this inoculum is already on the grain at harvest, uniformly distributed but mostly below the level of detection of usual isolation methods. More is added during combine harvesting and drying and when it is placed in store. Dust, a constant problem in grain stores, is largely composed of fungus and actinomycete spores and bacteria.

V. Fungal development during storage

The growth of fungi in stored grain is largely governed by temperature, the availability of water in the substrate and intergranular gas composition. However, the effects of these factors may be modified by ventilation, damage to the grain, the presence of foreign material, insect and mite infestations and chemical preservatives.

VI. Temperature

All fungi have characteristic temperature requirements for growth. Most thrive at 25 to 30°C but temperatures at which they grow range from -4 to 35°C for *Penicillium aurantiogriseum* to 30 to 60°C for *Humicola lanuginosa* and 12 to 55°C for *Aspergillus fumigatus*. Sporulation occurs over a similar range to growth but may be slow near the limits. The temperature of stored grain may be modified by spontaneous heating if heat is produced by respiration faster than it can escape. Spontaneous heating in barley occurs above 0.9 a_w (20% water content) and the maximum temperature attained increases with a_w to a maximum of about 65°C at $1.0a_w$ about 40% water content (Hill & Lacey 1983b).

II. Water availability

Water content is commonly used to express the amount of water in a substance but it gives little idea of the availability of the water for microbial growth since the relationship between water content and water availability differs between different types of seed. A better measure is given by a_w (the ratio of the vapour pressure of water over the substance to that of pure water at the same temperature and pressure).

Fungi differ widely in their tolerance of low a_w. Most field fungi require >0.90 a_w, although *Alternaria alternata* and *Cladosporium herbarum* can grow at 0.88 a_w. Many *Aspergillus* and *Penicillium* species are tolerant of lower a_w with *P. brevicompactum* growing down to 0.82 a_w and *Eurotium amstelodami* to 0.72 a_w. Spore germination may occur at slightly lower a_w than growth, while sporulation occurs within narrower limits. For safe storage, a_w should be less than 0.65.

III. Interaction of temperature and water activity

Temperature and water activity interact to determine the range of conditions over which individual fungal species can grow. Changing either factor affects the growth rate and may affect the ability of a fungus to compete with other species. The conditions under which fungi are most numerous are not necessarily those at which they grow best. Often, these are conditions under which they survive better than other species. Thus, although optima for growth of *Penicillium aurantiogriseum* and *P.viridicatum* are about 25°C and 1.0 a_w and for *Eurotium* spp. (*Aspergillus glaucus* group) about 30°C and 0.9 a_w, the penicillia are most abundant close to 0°C and 1.0 a_w and *Eurotium* spp. at about 30°C and 0.7 a_w. At higher temperatures and a_w, respectively, they are replaced by other species (Fig. 1). Storage history can, to a large extent, be determined from the predominant species, especially of *Aspergillus*, in a sample of grain (Hill & Lacey 1983b, Magan & Lacey 1984a,b).

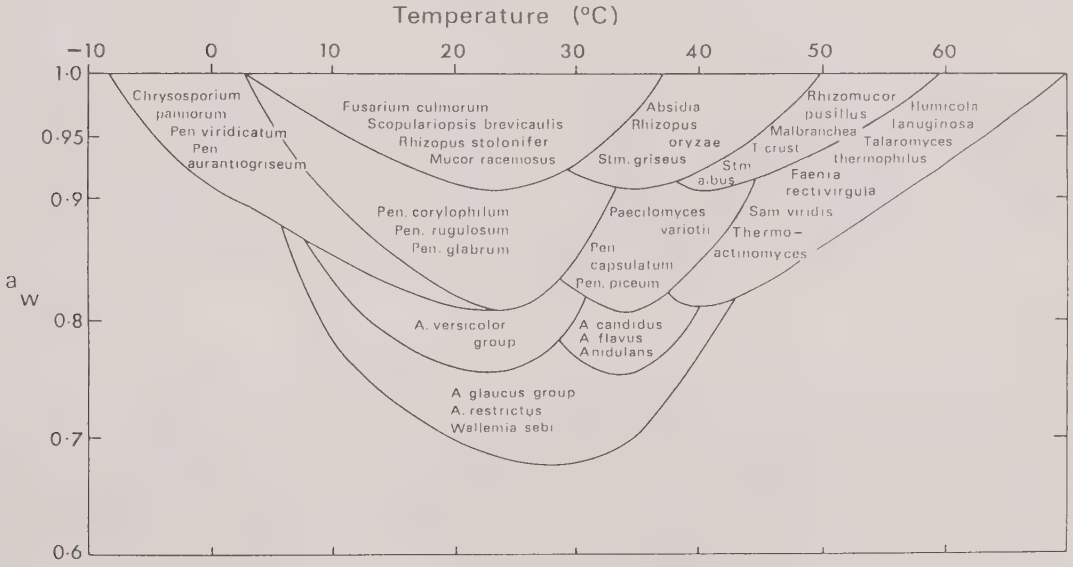

Fig. 1. Limiting conditions of temperature and a_w for growth of storage microorganisms

X. Gas composition

$C0_2$ is produced by respiration of both the grain and its microflora and can accumulate within grain bulks to form $>60\%$ of the intergranular atmosphere with 0_2 decreasing to $<1\%$. This is often sufficient to inhibit fungal growth, a principle exploited to store damp grain safely. Fungi differ in their ability to tolerate changes in the intergranular atmosphere (Table 2). The growth rates of *C. herbarum* and *P. aurantiogriseum* at 23°C are halved by decreasing 0_2 to 0.7% and 0.6%, respectively, or by increasing $C0_2$ to 13% and 4.5% (Magan & Lacey, 1984c). However, the $C0_2$ figure for *P. aurantiogriseum* is perhaps spurious because growth was stimulated by greater concentrations of $C0_2$. *Penicillium roquefortii* is one of the most tolerant filamentous fungi since its growth rate is halved only when 0_2 is decreased to $<0.14\%$ and it can still grow with 75% $C0_2$ (Golding 1940). $C0_2$ and 0_2 concentrations may interact so that fungi are more sensitive to high $C0_2$ when 0_2 is low than

when it is at normal levels. Tolerance of adverse gas composition also interacts with a_w and temperature so that tolerance of low 0_2 and high $C0_2$ concentrations often decreases as both depart from optimum levels.

The combined effects of a_w, temperature and gas composition can be seen in sealed and unsealed silos used to store damp, barley grain (Lacey 1971, Clarke & Hill 1981). While $C0_2$ levels are high fungi are few. As air leaks into sealed silos or as rate of removal of grain from the exposed surface in unsealed silos declines, first yeasts, especially *Hyphopichia burtonii* and *Hansenula anomala* become abundant, then *Penicillium* spp., especially *P. roquefortii*. With more aeration, spontaneous heating commences and these species are replaced first by *P. rugulosum* and *Eurotium* spp. and then *A. candidus*. With heating to 30-35°C, *Absidia corymbifera* and *Rhizomucor pusillus* predominate *Aspergillus fumigatus* appears at about 40°C and *Humicola lanuginosa* with thermophilic actinomycetes at 50 60°C.

Table 2. *Concentrations of oxygen and carbon dioxide required to halve linear growth of field and storage fungi at 23 and 14°C*

Temperature	23°C			14°C		
a_w	0.98	0.95	0.90	0.98	0.95	0.90
Oxygen				(per cent)		
Alt. alternata	2.80	<0.14	0.14	0.60	3.80	5.00
C. herbarum	0.70	5.20	10.00	N.D.	N.D.	N.D.
F. culmorum	<0.14	2.60	<0.14	9.90	12.50	5.00
P. a'griseum	0.60	5.30	2.40	0.50	<0.17	10.20
Asp. fumigatus	3.40	5.40	6.20	N.D.	N.D.	N.D.
E. repens	0.60	3.00	5.00	0.85	0.90	4.00
Carbon dioxide						
Alt. alternata	>15.0	>15.0	>15.0	>15.0	>15.0	4.5
C. herbarum	13.0	>15.0	>15.0	N.D.	N.D.	N.D.
F. culmorum	14.0	13.5	>15.0	8.0	11.5	14.0
P. a'griseum	4.5*	4.0*	>15.0	11.0	12.0	8.0
Asp. fumigatus	>15.0	5.2	12.5	N.D.	N.D.	N.D.
E. repens	>15.0	>15.0	>15.0	>15.0	9.0	>15.0

Key to abbreviations: *Alt., Alternaria; Asp., Aspergillus; C., Cladosporium; E., Eurotium; F., Fusarium; P. a'griseum Penicillium aurantiogriseum;* *, stimulation of growth by greater CO_2 concentrations.

X. Ventilation

Ventilation of grain with ambient or warmed air removes heat, and $C0_2$, introduces 0_2 and removes o introduces moisture, depending on the ambient relative humidity and the a_w of the grain. It thu upsets any equilibrium that had been reached in the grain bulk, causing the internal microflora t experience conditions different from those at the seed surface. How this affects growth of fungi ha hardly been studied but sporulation on the surface could be prevented while conditions within th seed are still suitable for growth.

XI. Fungal interactions

Colonization of the new substrate depends on the ability of a fungus to compete with the existin microflora and to withtand subsequent invasions. A range of interactions between pairs of organism has been recognised, including neutralism where neither benefits; commensalism and mutualism where, respectively , one or both benefit; amensalism where one is inhibited with no effect on th other; and competition where growth of both is decreased through common dependence on the sam substrate. Such interactions may vary with substrate, a_w and temperature. In natural conditions it i often difficult to determine whether the whole surface is equally susceptible to colonization and t what extent antimicrobial substances are produced. Nevertheless, principal component analysis o the variables affecting populations on ripening wheat suggests that there is spatial competition for th same niche between *Alternaria, Cochliobolus* and *Acremonium* and between *Cladosporium* an *Epicoccum*, and antagonism between the two groups and between *Alternaria* and *Fusarium*. How ever, in store, all storage fungi had a detrimental effect on *Alternaria* but they did not affect on another, perhaps because they had different a_w and temperature preferences (Wallace & Sinha 1981 1983). By contrast, Magan & Lacey (1984b) found considerable differences in the competitivenes

of storage fungi on agar, with *Penicillium hirsutum* and *Aspergillus candidus* among the most competitive. Competitiveness was usually less on wheat extract agar than on richer malt extract agar and sometimes differed on wheat grain where *A. versicolor* was often dominant (Magan & Lacey 1985).

XII. Grain damage

Damaged grain is much more susceptible to fungal invasion and also shows more visible moulding (Welling 1968, Perez *et al.* 1982). The increase in susceptibility depends on the nature of the damage, the genotype of the grain and the period of storage. Injury to barley grain increases mycelial growth on the grain; *Penicillium* spp. become dominant on injured grains while *Aspergillus* spp. are unaffected by the degree of injury. With maize, damage to the germ increases moulding most. Although severe injuries have a disproportionate effect on resistant varieties, varietal differences are maintained. Resistance to seed breakage could thus be an important factor in determining resistance to moulding (Tuite *et al.* 1985).

XIII. Foreign material

When filling grain stores, foreign material composed of grain and plant fragments, dust, weed seeds, insect debris and soil may become concentrated in zones. For instance, fine material may settle at the outsides when filling bins by auger. Such material may differ from the grain in a_w, interfere with airflow during ventilation and be more susceptible to fungal invasion, providing a focus of infection for the surrounding grain.

XIV. Insects and mites

Infestation of grain by insects and mites damages the grain, introduces fungal inoculum and, through respiration, causes heating and increases a_w. Heating to $>40°C$ may occur even in dry grain. Insect infestation and fungal colonization are closely interrelated: for instance, *Acarus siro* can feed and breed on *Penicillium* spp., especially *P. aurantiogriseum*. By contrast, *Cryptophagus* beetles may deter *Rhizopus*, although it is enhanced by *Cryptolestes* which feeds on the seed embryo. Insect excreta may in turn be colonized by *Streptomyces* while uric acid hastens the disappearance of *Alternaria* and growth of *Aspergillus* (Sinha *et al.* 1972, Wallace & Sinha 1981).

XV. Period of storage

Storage time interacts with all other factors to determine the degree of moulding. Consequently, the longer grain has to be stored, the drier or cooler it should be. Prolonged storage of grain may also affect the susceptibility of the grain to fungal growth (Perez *et al.* 1982). Maize containing up to 15.5% water could be stored for at least 7.5 months at ambient Indiana (USA) postharvest temperatures without visible moulding while that containing 18.0% water had visible moulding on broken kernels, although all was invaded by *Penicillium* spp. and members of the *Aspergillus glaucus* group. Grain transferred to 26°C and 84% relative humidity moulded more readily after storing with $<15.5\%$ water content for 7.5 months than grain stored for <5.5 months.

Fungi can survive long periods in grain stored at low water contents. Wheat at ambient temperatures with $<12.6\%$ water content yielded *Alternaria* spp. for up to 8 years but *Cladosporium* and *Verticillium* spp. for <4 years. Grains yielding *Penicillium* spp. also declined by 90% within 6 years but those yielding *Aspergillus* spp. increased from 17-18 to 42-47% in 10 years. Field fungi survived longer at 4.5°C than at 25°C but storing with 2% 0_2 did not affect survival (Pixton *et al.* 1975). At 14% water content *Alternaria* spp. survived in barley for only 6 months although storage fungi only increased substantially above 16% water content (Lutey & Christensen 1963).

XVI. Chemical preservatives

Most chemicals used to preserve damp grain are fungistatic rather than fungicidal. Consequently, propagules of storage microorganisms remain viable and can grow if conditions again become suitable. Some tolerate larger concentrations of preservative than others and can metabolize it when doses are inadequate for the water content of the grain or when application is uneven. With propionic acid, *Paecilomyces variotii* and *Eurotium* spp. tolerate up to 1% in agar media when other species are inhibited by $<0.2\%$. Such tolerant species may then dominate the microflora, at least temporarily (Lord *et al.* 1982) but once the preservative is metabolized, less tolerant species may be able to grow.

XVII. Geographical location

The pattern of fungal colonization varies little with location but there are differences in detail. In tropical climates, additional species may be found on the grain, especially before harvest. These include not only such genera as *Curvularia* and *Nigrospora* but also species considered as storage fungi in temperate climates, e.g. *Aspergillus flavus* (Hill *et al.* 1985). In stored grain, *Penicillium* spp. tend to predominate in temperate regions and *Aspergillus* spp. in tropical and subtropical areas, accompanied by *Penicillium* and *Rhizopus* in the humid tropics, and bacteria if the grain is stored wet (Sinha 1973). Although it is usually easier to store grain well in dry climates, unusual storage species sometimes occur, e.g. *Aspergillus aurantiobrunneus* and *A. egyptiacus* in grain from Iran. However, these are broad generalisations. The microflora in individual samples will depend on grain type and harvesting methods, a_w, drying and storage practice.

References

Clarke, J.H.; Hill, S.T. (1981) Mycofloras of moist barley during sealed storage in farm and laboratory silos. *Transactions of the British Mycological Society* **77**, 557-565.

Golding, N.S. (1940) The gas requirements of molds. III. The effect of various concentrations of carbon dioxide on the growth of *Penicillium roquefortii* in air. *Journal of Dairy Science* **23**, 891-898.

Hill, R.A.; Lacey, J. (1983a) The microflora of ripening barley grain and the effects of pre-harvest fungicide application. *Annals of Applied Biology* **102**, 455-456.

Hill, R.A.; Lacey, J. (1983b) Factors determining the microflora of stored barley grain. *Annals of Applied Biology* **102**, 467-483.

Hill, R.A.; Wilson, D.M.; McMillan, W.W.; Cole, R.J.; Sanders, T.H.; Blankenship, P.D. (1985) Ecology of the *Aspergillus flavus* group and aflatoxin production in maize and groundnut. In *Trichothecenes and other mycotoxins* (J. Lacey, ed.), 79-95. Chichester; John Wiley.

Joffe, A.Z. (1965) Toxin production by cereal fungi causing toxic alimentary aleukia in man. In *Mycotoxins in foodstuffs* (G.N. Wogan, ed.), 77-85. Massachusetts; M.I.T. Press.

Lacey, J. (1971) The microbiology of moist barley storage in unsealed silos. *Annals of Applied Biology* **69**, 187-212.

Lord, K.A.; Lacey, J.; Cayley, G.R. (1982) Laboratory tests of 8-quinolinol as an additive to volatile fatty acids for preserving hay and other feedstuffs. *Animal Feed Science and Technology* **7**, 287-302.

Lutey, R.W.; Christensen, C.M. (1963) Influence of moisture content, temperature and length of storage upon survival of fungi in barley kernels. *Phytopathology* **53**, 713-717.

Magan, N.; Lacey, J. (1984a) Effect of temperature and pH on water relations of field and storage fungi. *Transactions of the British Mycological Society* **82**, 71-81.

Magan, N.; Lacey, J. (1984b) Effect of water activity, temperature and substrate on interactions between field and storage fungi. *Transactions of the British Mycological Society* **82**, 83-93.

Magan, N.; Lacey, J. (1984c) Effects of gas composition and water activity on growth of field and storage fungi and their interactions. *Transactions of the British Mycological Society* **82**, 305-314.

Magan, N.; Lacey, J. (1985) Interactions between field and storage fungi on wheat grain. *Transactions of the British Mycological Society* **71**, 29-37.

Marasas, W.F.O.; Kriek, N.P.J.; Wiggins, V.M.; Steyn, P.S.; Towers, D.K.; Hastie, T.J. (1979) Incidence, geographic distribution and toxigenicity of *Fusarium* species in South African corn. *Phytopathology* **69**, 1181-1185.

Mills, J.T.; Frydman, C. (1980) Mycoflora and condition of grains from overwintered fields in Manitoba, 1977-78. *Canadian Plant Disease Survey* **60**, 1-7.

Perez, R.A.; Tuite, J.; Baker, K. (1982) Effect of moisture, temperature and storage time on subsequent storability of shelled corn. *Cereal Chemistry* **59**, 205-209.

Pixton, S.W.; Warburton, S.; Hill, S.T. (1975) Long-term storage of wheat. III. Some changes in the quality of wheat observed during 16 years of storage. *Journal of Stored Product Research* **11**, 177-185.

Sinha, R.N.; Van Bronswick, J.E.M.H.; Wallace, H.A.H. (1972) Canonical correlation analysis of abiotic and biotic variates in insect-infested grain bulks. *Oecologia* **8**, 321-333.

Sinha, R.N. (1973) Ecology of storage. *Annales de Technologie Agricole* **22**, 351-369.

Sutton, J.C. (1982) Epidemiology of wheat head blight and maize ear rot caused by *Fusarium graminearum*. *Canadian Journal of Plant Pathology* **4**, 195-209.

Tuite, J.; Koh-Knox, C.; Cantone, F.A.; Bauman, L.F. (1985) Effect of physical damage to corn kernels on the development of *Penicillium* species and *Aspergillus glaucus* in storage. *Phytopathology* **75**, 1137-1140.

Wallace, H.A.H.; Sinha, R.N. (1981) Causal factors operative in distributional patterns and abundance of fungi: a multivariate study. In *The fungal community: its organization and role in the ecosystem* (D.T. Wicklow; G.C. Carroll, eds), 233-247. New York; Marcel Dekker.

Wallace, H.A.H.; Sinha, R.N. (1983) Biological, physical and chemical changes in stored wheat. *Mycopathologia* **82**, 65-76.

Welling, B. (1968) The influence of thresh damage on microflora and germination of barley during storage. *Tidsskrift for Planteavl* **72** 513-519.

poilage and Mycotoxins of Cereals and other Stored Products.
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The Microbial Content of Wheat and Flour

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. Summary

To assist the miller who wishes to produce 'low count' flours, a series of investigations has been undertaken to determine the factors affecting the load of microorganisms in wheat and flour. The weather conditions prevailing during growth and harvesting are shown to be of primary importance in governing the numbers of microorganisms present in wheat and hence the resultant flour. Of the various types of screenroom apparatus examined, the use of separators to select high specific gravity grain and scourers to clean the grain are most likely to reduce microbial counts. The numbers of bacteria and moulds in wheat and flour reduce during storage, particularly at elevated temperatures.

I. Introduction

In recent years there has been an increasing demand for the miller to supply special flours conforming to set microbiological specifications. These special flours are required for canned foods, where the specifications for the number of thermophilic bacteria present are stringent, for lightly processed or unprocessed baby foods where food poisoning bacteria must be absent and the general level of contamination must be low, and for a wide variety of the foods such as refrigerated dough products, pie pastry, sausages, pasta products, etc., where low counts of bacteria and moulds are required.

In order to assist the miller in producing such 'low count' flours investigations have been carried out to obtain a better understanding of the factors influencing the load of microorganisms in wheat and flour and the effects of the various cleaning processes taking place at the mill in reducing this load.

III. Contamination of wheat in the field

During 1976 and 1977 tests were carried out to determine the numbers of bacteria and fungi on wheat during development in the field up to the time of harvest. The average counts in the wheat at various times during the growing season are given in Fig. 1 for bacteria and Fig. 2 for moulds.

There was a considerable difference between the growing conditions in 1976 and 1977. In the former year the weather was favourable and the wheat had reached growth stage 10 (late boot, just prior to emergence of ear) by late May and was harvested in early August, whereas in 1977 unusually wet conditions prevailed and the wheat did not reach growth stage 10 until the end of June and was not harvested until September. The microbial counts reflect the difference between the conditions in

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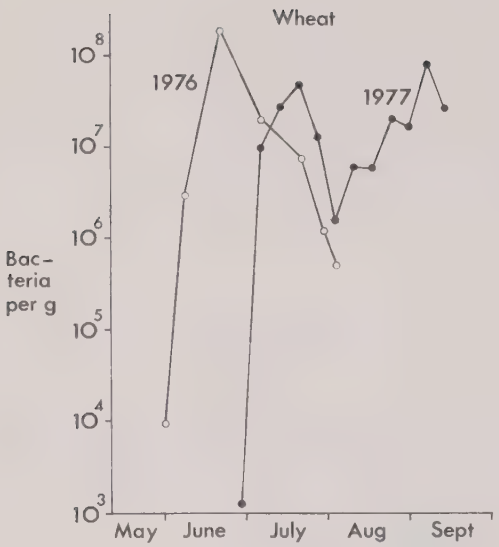


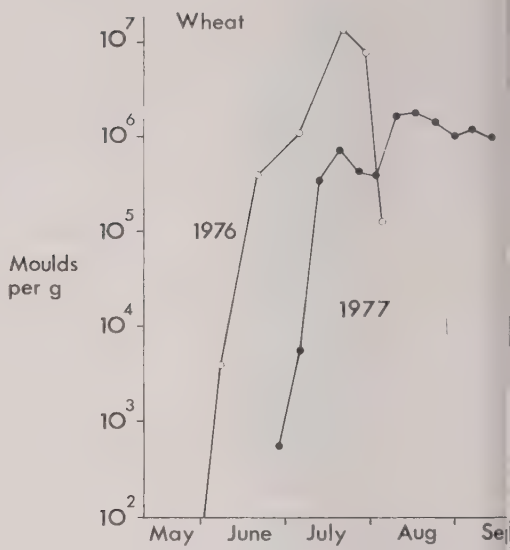


Fig. 1. Bacterial counts in wheat during the growing season
Fig. 2. Mould counts in wheat during the growing season

the two years of test. In 1976, the bacterial count reached a peak of 200 million per g in late June and decreased steadily thereafter under the warm dry conditions which prevailed until the count had reduced to 0.5 million per g. The pattern was similar with moulds except that the peak count of 10 million per g was reached rather later. By contrast, in 1977 counts started to decrease during the early part of summer, when the weather was largely fine, but increased again during the wet conditions which prevailed in late summer, so that at harvest very high counts of 125 million bacteria and 1 million moulds per g of wheat were obtained.

It is clear from these results that count of microorganisms in wheat can be greatly influenced by the weather conditions which, unfortunately, are beyond man's control. No doubt other factors such as the type of soil in which the wheat is grown, the use of fertilisers, crop sprays, etc., will also affect the numbers and types of microorganisms present but none of these is likely to be as important as the weather.

IV. Contamination during harvest

Although modern methods of harvesting have reduced the incidence of contamination of wheat with soil, it is inevitable that this will still occur to some extent, particularly where the crop has lodged. Soil particles have a very high microbial count and, moreover, may contain specific organisms of importance, such as thermophilic and mesophilic sporing bacteria and coliforms, which are not naturally present in the wheat.

The harvested grains at the farm may also be subject to adventitious contamination. For example, particularly on mixed farms, the floors on which the wheat is deposited may not be in a clean condition and at worst could be contaminated with manure. In these circumstances the wheat can pick up bacteria of faecal origin, such as *Salmonella*.

V. Distribution of microorganisms on the grain surface

The results from two tests comparing counts on dissections from the epidermis of the back, beard and crease of 50-100 grains of the same sample of wheat are shown in Table 1.

In both tests the beard is shown to have the highest count and the crease the lowest count. The low count in the crease is unexpected since it has always been assumed that this is a major site of contamination.

Table 1. *Distribution of bacteria on the surface of grain*

Epidermal fracton	Total bacteria per g.	
	Test 1	Test 2
Back	51,500	295,000
Beard	267,000	670,000
Crease	19,500	38,500

I. Effect of screenroom operations

In the screenroom of a mill there are various pieces of machinery which serve either to separate foreign matter such as straw, stones, soil and seeds from wheat or to clean the wheat to remove more firmly attached soiling. Some of systems used are listed in Table 2.

Table 2. *Separation and cleaning systems used in the screenroom of mills*

Separating systems	Cleaning systems
Milling separator	Scourer
Dry stoner	Entoleter/aspirator
Disc separator	Brush
Aspirator	
Specific gravity table	

The effects of these systems in reducing the numbers of microorganisms present on the wheat are discussed separately.

Milling separator, stoner, discs, aspirator

There is no evidence to suggest that any marked reduction in count occurs as a result of passing the wheat through these separating systems. In fact, with the aspirator, it is possible that the count may be increased if the distribution and velocity of the air used is not sufficient to remove all the high-count dust particles.

II. Specific gravity tables

These machines separate the wheat into various fractions according to specific gravity. The results from tests in which the bacterial counts were determined in wheat and the resulting straight run flour from each of five fractions separated on two different types of table are shown in Fig. 3.

It is apparent that, with both types of table, the counts in wheat and flour increase as the specific gravity of the fraction decreases. Based on these results it could be expected that flour made from wheat from the high specific gravity fractions 1, 2 and 3, which comprise over 90% of the total grain, would have bacterial counts more than 50% lower than flour made from all fractions. Unfortunately, the capacity of these tables is small and a large number would be required to serve the average-sized mill.

III. Scourers and brushes

During visits to mills opportunity has been taken to examine the effects of a variety of different dry scourers, entoleter/aspirators and brushes in reducing counts of bacteria and moulds in wheat. The results for bacterial counts are summarised in Table 3.

The majority of the dry scourers examined caused a small reduction in count of both bacteria and moulds in wheat. On no occasion was a marked increase in count obtained as a result of treatment. Essentially similar results were obtained with the entoleter/aspirators.

Brushes were used in most screenrooms as a final cleaning operation after the wheat had been conditioned and just before entering the millroom. In most cases a reduction in count, sometimes substantial, was achieved, but at several mills very much higher counts of both bacteria and moulds

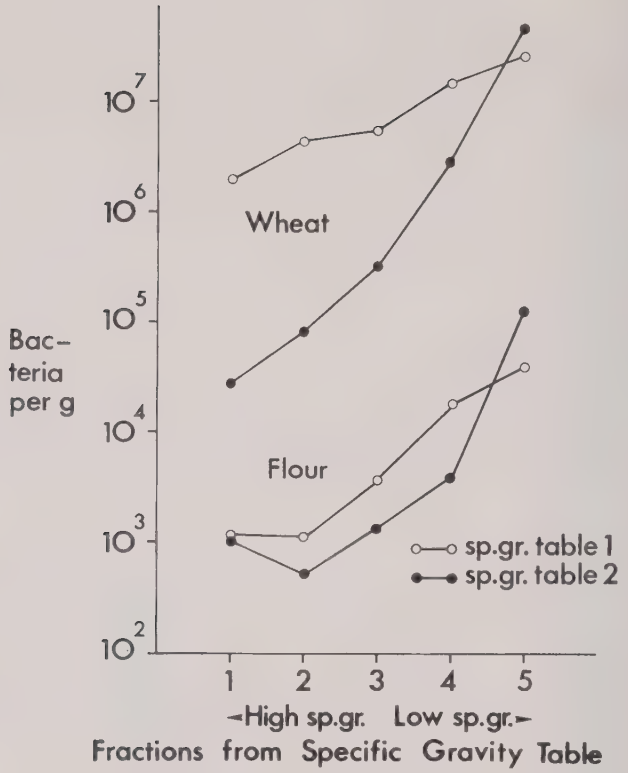


Fig. 3. Bacterial counts in wheat fractions of varying specific gravity, and in the flour milled from them.

Table 3. *Effect of screenroom scourers and brushes on counts of bacteria in wheat*

Cleaning System	No. of tests	Results of treatment count increased	count reduced
Dry scourer	7	2	5
Entoleter/aspirator	12	3	9
Brush	12	4	8

were obtained from the wheat as a result of brushing. In one case the bacterial count was increased by 400% and in another the mould count was increased by over 1000%.

IX. Chlorination of the tempering water

In order to improve the microbiological quality of wheat and hence flour it is the practice at some mills to add 50-200 ppm of chlorine to the water used for conditioning the wheat prior to milling. Only one of the mills visited on the course of these investigations used chlorine and tests showed that the bacterial count was reduced by 78% as a result of the treatment. Information on the effects of chlorination is limited and this aspect requires further investigation.

The results from this survey of the various separation and cleaning operations taking place in the screenroom of the mill suggest that the most worthwhile reductions in counts are likely to be obtained by selection of high specific gravity grain, the use of scourers and, perhaps, chlorination of the tempering water. The evidence indicates that aspirators and brushes are best omitted from the screenroom activities unless they are well maintained and regularly cleaned.

X. Relationships between counts in wheat and flour

In one investigation samples of wheat and the resultant flour obtained from three different mills at regular intervals over seven months were tested for counts of bacteria and moulds. The mean log counts are given in Table 4.

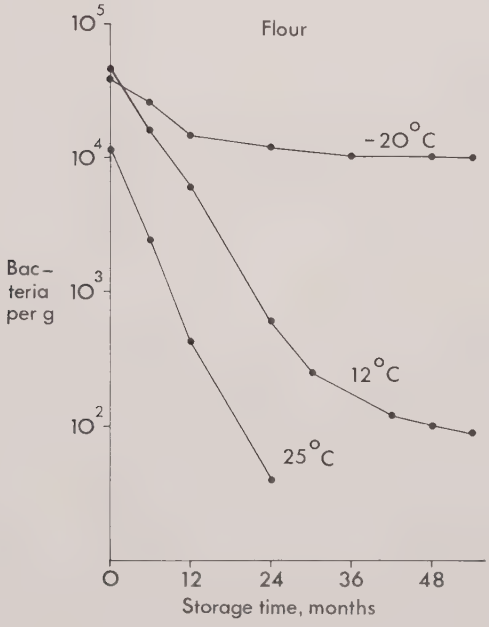
Table 4. *Mean log counts of microorganisms in wheat and flour samples taken from three mills*

Mill	Log count per g			
	Bacteria		Moulds	
	Wheat	Flour	Wheat	Flour
A	6.42	4.46	4.36	3.71
B	6.44	4.57	4.33	3.58
C	6.47	4.64	4.20	3.28

The average counts at the three mills were remarkably similar. It is interesting to observe that the bacterial counts in flour are about 100 times less than in the corresponding wheat whereas the mould counts are only about 10 times less. The reason for this is not clear. Perhaps the moulds are present in the deeper tissues of the epidermis of the grain and are less readily removed by the screenroom cleaning procedures.

XI. Effect of storage

The numbers of microorganisms present in wheat and flour tend to decrease during storage, the rate depending on moisture content and storage temperature. The effect of storage at -20°C, 12°C and 25°C on the change in counts of bacteria and moulds in flour of moisture content 12-13% is shown in Fig. 4 and 5, respectively.



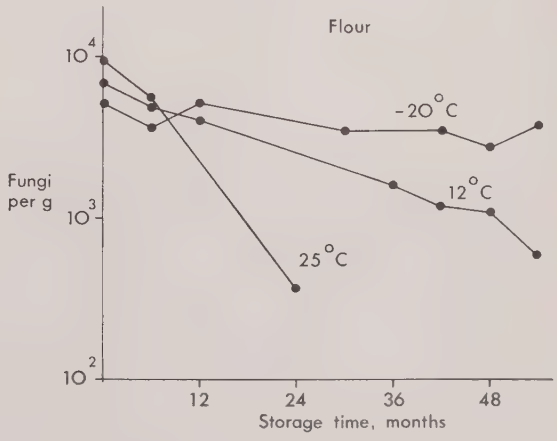


Fig. 4. Bacterial counts in flour stored at different temperatures
Fig. 5. Mould counts in flour stored at different temperatures

Little change in count occurs during prolonged storage under deep freeze conditions (-20°C) but at 12°C and more so at 25°C there is a steady reduction in numbers with storage time. The mould counts (Fig. 5) reduce more slowly than the bacterial counts (Fig. 4). Similar results can be expected with wheat. At increased moisture contents the reduction in bacterial counts during storage will tend to be greater but at a moisture content of 15% or above there is a danger of mould growth.

XII. Conclusions

The fact that microbial numbers in wheat are so dependent on the weather conditions means that the miller supplying special 'low count' flours is likely to have more trouble in meeting specifications in some years than in others. However, even in a year where the growing and harvest conditions are good, the counts in wheat and hence flour are likely to be variable, depending on the methods of harvesting and storage time before receipt. The investigations described in this paper suggest that there are a number of steps which could be taken to improve the situation. One approach is to select wheats of suitable microbiological quality. This requires that facilities are available to carry out the necessary tests, which is not the case at most mills. In a bad harvest year suitable wheats might be imported from abroad. However, such wheats can be expensive and there is no guarantee that the counts will necessarily be any lower than in the UK wheat. A second approach is to carry out a critical appraisal of the effects of the various screenroom activities. The results from this investigation suggest that selection of high specific gravity grain cleaned with scourers and treated with chlorine prior to conditioning may produce the desired reduction in counts.

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Spoilage and Mycotoxins of Cereals and other Stored Products.
Edited by B. Flannigan

Bacterial and Mould Spoilage in Coconut

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I. Summary

Microbial associations are responsible for the production of off-odours in coconut. At low water activities methyl ketones, secondary alcohols and free fatty acids are produced by fungal growth. At higher water activities a bacterial association is responsible for the production of 2,3-butanediol and buttery off-odours.

II. Introduction

Spoilage represents a detrimental change in smell, taste or texture of a food rendering it unfit for consumption. These changes may be due to microbial contamination or autocatalytic processes including oxidation. Mossel & Ingram (1955) in their classic review on the physiology of microbial spoilage consider the conditions under which spoilage can occur. They define extrinsic, intrinsic and implicit factors which include the environment, the food substrate and the particular microflora. The shelf life of a food is the time taken for an unacceptable organoleptic change to be detectable.

Food which is unfit is regularly discolored, friable or slimy, and frequently has an unpleasant off-odour. These characteristics reflect the breakdown of insoluble proteins, carbohydrates and fats by microbial action to give low molecular weight compounds, some of which are volatile and contribute to an off-odour. Some are water-soluble and contribute to a loss of texture, whilst others may be pigmented and contribute to discoloration.

Food may be treated to extend its shelf-life; treatments include high or low temperature processes, irradiation, removal of water or oxygen, packing with increased carbon dioxide levels or the addition of chemicals such as preservatives, alcohol, acids or antioxidants (Gould *et al.* 1983). These factors are designed to extend the storage period either by a reduction in the numbers of micro-organisms present or by inhibition of their growth. The food is stable as long as the environmental factors do not permit microbial growth. If, however, these conditions change, then spoilage may take place owing to growth of some of the surviving organisms. This paper deals with the microbial content of coconut, as well as some of the specific chemical changes which occur during spoilage in desiccated coconut and in the commercial consumer returns which exhibit off-odours. These provide examples of spoilage of oil-rich, dried foods.

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III. Production, Use and Composition of Coconut Oil

Coconut oil and copra are the main exports of many Third World countries. The Philippines is the largest producer of both (over 75% of the total), but substantial quantities are produced throughout South-East Asia and the Pacific Islands. Spoilage may have substantial effects on these economies.

The fatty acid composition of coconut oil is unusual for two reasons. Firstly, approximately 60% of the constituent fatty acids are short chain (C_6-C_{14}), and secondly the proportion of the unsaturated fatty acids is extremely low (Table 1), making the oil relatively resistant to oxidative changes (Young 1983). The fatty acid composition gives the oil its characteristic properties, so that approximately half the annual production is used for technical purposes such as the manufacture of soaps and detergents, as well as body and sun tan lotions. The rest is used in foods such as milk substitutes, filled milk, ice cream and topping, as shortening and fillings for biscuits, as a component of margarine and in the production of confectionery products (Young 1983, Kinderlerer 1984a).

IV. Process Conditions

Desiccated coconut is heated twice during its manufacture, firstly when pieces of coconut meat are immersed in boiling water for not less than 1.5 min, and secondly when the shredded coconut is dried in hot air for 20-30 min at 93°C before it is packaged (CAC Recommended Codes of Practice, 1971). Its final water activity is 0.32-0.42; at this level it is stable and will not spoil although bacteria and moulds are present (Kinderlerer & Clark 1986).

Table 1. *Fatty acid composition of edible coconut oil [Codex Alimentarius Commission FAO/WHO 79/17, cited by Young (1983)]*

Fatty Acid	Composition (%)
6:0	<1.2
8:0	3.4–15
10:0	3.2–15
12:0	41 –56
14:0	13 –23
16:0	4.2–12
18:0	1.0–4.7
18:1	3.4–12
18:2	0.9–3.7

V. Bacteriology and Bacterial Spoilage

The major work on desiccated coconut in the 1960's was concerned with the presence of *Salmonella* (Semple *et al.* 1961). General improvements in hygiene in the coconut mill have reduced the incidence of this pathogen (Roberts *et al.* 1982). Bacteria, moulds and yeasts are often present in coconut meat and the main bacterial genera found by Kajs *et al.* (1976) were *Flavobacterium, Microbacterium, Micrococcus* and various members of the Enterobacteriacae.

A small microbiological survey has been made of four different types of Sri Lankan desiccated coconut: purchased from supermarkets; obtained directly from processing and packaging companies; commercially pasteurised (steam at 92°C for 8 min); and material which was returned to one manufacturer as spoilt and rancid (Table 2). The consumer returns had the lowest aerobic plate count (0.61×10^3 per gram) of all the samples, even though they showed signs of spoilage and discoloration. This was in contrast to material from supermarkets and food packers where the counts ranged from 10^3 to 10^5 colony forming units per gram. Generally, consumer returns contain Gram-positive spore-forming bacilli whilst normal coconut contains Gram-positive cocci. Pasteurisation with steam (92°C for 8 min) causes a reduction in the aerobic plate count by a factor of between 3 and 10. Low numbers of *Staphylococcus aureus*, of which a small proportion are coagulase-positive, could be transferred to the coconut by the food handler (the testa is removed by hand) and might become the foci of food-borne infection (Kinderlerer & Clark 1986).

Some desiccated coconut displays a sour buttery odour (D.M. Busfield, personal communication). A similar odour developed when desiccated coconut, sterilised at 121°C for 15 min, was stored at 25°C and a water activity of 0.90 for two months. No mould growth was visible. The total aerobic count after storage was 10^5 colony forming units per gram, the dominant organism was *Bacillus*

Table 2. *Viable count of aerobic micro-organisms in dessicated coconut (3 days at 25°C).* (Kinderlerer & Clark, 1986)

Company	Source	No. of analyses	Mean count/g $\times 10^3$	S.D.
A	Supermarket	39	3.34	1.24
B	Supermarket	41	80.20	52.00
C	Supermarket	20	13.10	6.70
D	Supermarket	20	333.00	116.00
E	Food processor	21	40.00	11.20
E′	Food processor	23	33.20	14.40
F	Food packer	25	3.24	1.58
F′	Food packer	16	66.30	19.70
C′	Food packer	15	26.30	11.70
G	Food processor (pasteurised)	31	1.37	2.07
F″	Consumer returns	84	0.61	0.56

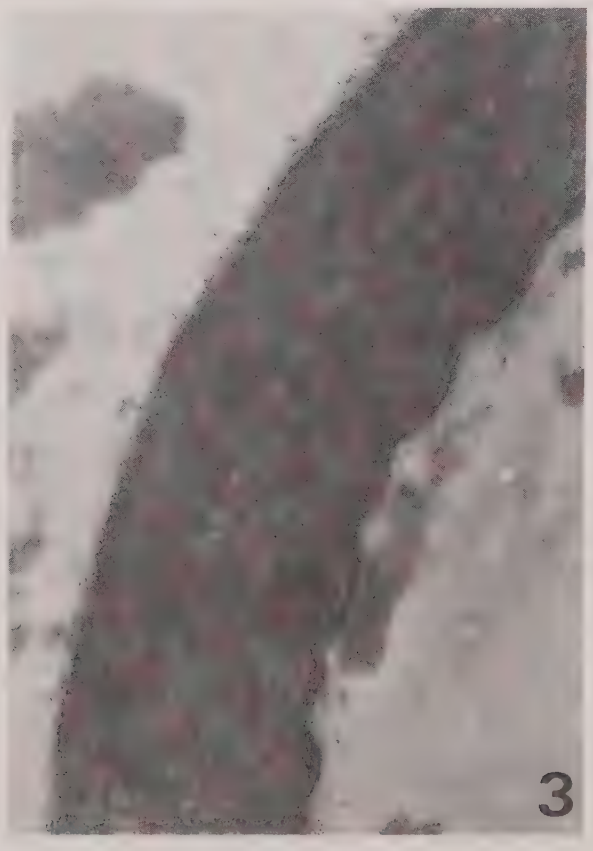
subtilis. The volatile compounds diacetyl, acetoin and butanediol, which all have a buttery odour similar to that found in some of the commercial consumer returns, were isolated from the stored material and identified by gas chromatography and mass spectrometry. Butanediol is a common product of bacterial fermentation formed by reduction of acetoin (Thimann 1967).

Many bacterial species, including *B. subtilis, Enterobacter aerogenes, Serratia marcescens* and *Streptococcus lactis* subsp. *diacetylactis* have been shown to metabolise labelled intermediates in the production of butanediol (Speckman & Collins 1981). *B. subtilis* is extremely heat resistant owing both to heat resistant endospores and the fact that it is a thermophilic organism capable of growth at 55°C (Bergey 1975). The low water activity and high oil content probably enhance the resistance of the bacteria to heat.

VI. The Mycoflora of Coconut and Concomitant Spoilage

Fungal spoilage has been known for a long time. Biffen (1899) suggested that amyl butyrate was produced by growth of a mould on coconut and commented on the ubiquitous presence of *Penicillium* and *Eurotium* species. Later Starkle (1924) and Stokoe (1928), working with coconut oil, showed that aliphatic methyl ketones were produced from short chain fatty acids by some species of *Aspergillus* and *Penicillium*. Other workers in the 1930's showed that a number of fungi isolated from coconut and copra had lipolytic activity (see Kinderlerer 1984a). The ability of fungi to hydrolyse triacylglycerols would account for the accumulation of free fatty acids in oil seeds which occurs after fungal spoilage (Eggins & Coursey 1968). Twenty-eight fungal species have been isolated and identified from Sri Lankan desiccated coconut (Kinderlerer 1984b). These moulds fall into three groups - field, storage and advanced decay fungi (Table 3). Fifteen known xerophilic species were isolated, some of which were isolated by preparing decimal dilutions in full-strength Ringer's solution, 1% peptone or 40% sucrose and surface plating onto high solute media such as malt salt, malt sucrose, dichloran 18% glycerol (DC18), malt yeast 40% glucose (MY40G) agars. Others were isolated by allowing the moulds to grow at low water activity in coconut using either added water or 20% glycerol. Members of the genus *Eurotium* (the perfect state of the *Aspergillus glaucus* group) were the most common species, followed by *Penicillium* spp. These species are the common storage fungi on grain (Christensen 1965). Rarer moulds such as *Eremascus* and *Chrysosporium xerophilum* were found. Some of the fungi may form mycotoxins although the storage conditions under which toxins may be produced in coconut are not known (Kinderlerer 1984b).

Samples of desiccated coconut returned to a packer in the UK due to rancidity had been stored in damp conditions. Some appeared mouldy, others were discoloured. Fig. 1 shows an electron micrograph of rancid material. The cellulose microfibrils have become distinct and diffuse at the edge of the cell wall and the middle lamella is not visible. There has been breakdown of the cellulose and pectins. Fig. 2 shows a section through fresh coconut where both the cellulose microfibrils of the primary cell wall and the middle lamella can be seen. Normal desiccated coconut (Fig. 3) looks similar to fresh coconut but as it undergoes two heat treatments the cell wall becomes distorted and crinkled. The microflora of the rancid material was extremely simple. A small bacterial population consisting mainly of Gram-positive bacilli and low numbers of *Penicillium verrucosum* var. *cyclopium* and *Eurotium herbariorum* were found (Kinderlerer & Clark, 1986).



Figs. 1, 2 and 3. Electron micrographs of part of the cell wall of rancid, fresh and non-rancid coconut (endosperm cell). Stained lead citrate (× 2600).

Table 3. *Fungi isolated from desiccated coconut* (Kinderlerer, 1984b)

Xerophilic or storage fungi:
Ascomycotina:
Eremascus albus Eidam
Eurotium amstelodami Mangin
Eurotium chevalieri Mangin
Eurotium herbariorum (Wiggers) Link ex Gray
Hyphomycetes:
Aspergillus flavus Link ex Fries
Aspergillus niger van Tieghem
Aspergillus penicillioides Spegazzini
Aspergillus restrictus Smith
Chrysosporium xerophilum Pitt
Paecilomyces variotii Bainier
Penicillium corylophilum Dierckx
Penicillium expansum Link ex Gray
Pencillium verrucosum var. *cyclopium* (Westling) Samson *et al.*
Penicillim spinulosum Thom
Field and advanced decay fungi:
Zygomcetes:
Absidia corymbifera (Cohn) Saccardo & Trotter
Rhizopus stolonifer (Ehrenb. ex Fries) Lind
Syncephalastrum racemosum Cohn ex Schroeter
Ascomycotina:
Chaetomium bostrychodes Zopf
Hyphomycetes:
Arthrinium phaeospermum (Corda) Ellis
Arthropsis spp. Sigler *et al.*
Asperigillus carneus (van Tieghem) Blochwitz
Botryosphaeria rhodina (Berk & Curt) von Arx
Botrytis cinerea Persoon ex Persoon
Cladosporium cladosporioides (Fres.) de Vries
Cladosporium sphaerospermum Penzig
Trichoderma koningii Oudem

VII. Storage of Desiccated Coconut at Low Water Activity

In an investigation on the keeping qualitites of desiccated coconut, Child (1941), working in New Guinea, found that coconut went rancid and mouldy when stored for two months if the water content exceeded 4.7%. To reproduce these results under controlled conditions desiccated coconut was stored for 14 weeks in sealed containers at water activities of 0.76 and 0.88 at 25°C. At the lower water activity a stable association of two xerophilic fungi, *E. amstelodami* and *P. citrinum*, was observed (Kinderlerer 1984a). This material had a rancid odour similar to that found in the consumer returns. At the higher water activity a less well defined spoilage occurred and the predominant mould was *Chrysosporium xerophilum*. In 1949, Westerdijk observed that a constant association of spoilage fungi developed under similar environmental conditions. Of the total microflora only a small group of organisms are capable of growth at low water activities.

VIII. Chemistry of Rancidity in Coconut

A number of volatile off-flavour compounds have been isolated from coconut oil and coconut meat. These include the aliphatic methyl ketones - $C_{7,9,11,13}$ (Stokoe 1928, Allen 1965, Lin & Wilkins 1970). In addition, pentadecan-2-one was found by Allen (1965) in coconut oil. Whilst the early work on spoilage in coconut oil was concerned with the production of methyl ketones (Stokoe 1928) the later work involved their synthesis in connection with the development of flavour in blue cheese by fermentation with *Penicillium roquefortii* (Kinsella & Hwang 1976).

The off-flavour compounds listed in Table 4 were obtained by vacuum distillation of oil extracted from the rancid consumer returns and from the material which was stored for 14 weeks at a water activity of 0.76.

Table 4. · *Volatile off-flavour compounds in rancid coconut oil*

Off-flavour compounds	Consumer return (mg/g oil)	Rancid coconut (mg/g oil)	Flavour note
Pentan-2-one	Trace	Trace	Pear drops
Pentan-2-ol	N.D.	0.5	Ethereal
Hexan-2-one	9.3	Trace	Ethereal
Hexan-2-ol	4.8	N.D.	Turpentine
Heptan-2-one	10.7	5.1	Rancid almonds
Heptan-2-ol	9.3	7.1	Rancid coconut
Octan-2-one	2.6	N.D.	Weakly ethereal
Nonan-2-one	19.6	3.0	Weakly turpentine
Nonan-2-ol	2.9	0.3	Musty, stale
Undecan-2-one	6.8	1.3	Weakly turpentine
Total	66.1	17.29	

An homologous series of methyl ketones ($C_{5,6,7,8,9,11}$) was identified, together with the corresponding secondary alcohols. These compounds have rancid, musty and stale odours somewhat similar to the rancid consumer returns. Other volatile compounds were isolated and identified in trace amounts, viz. butan-2-ol, hexanol, heptanol, octan-3-one, decan-2-one and tridecan-2-one. Similar series of compounds are found when *E. amstelodami, E. herbariorum, E. chevalieri* and *P. citrinum* are reinoculated into sterile coconut (Table 5). These results confirm that the methyl ketones and secondary alcohols are produced during the growth of some members of the normal mycoflora of coconut (Kinderlerer & Kellard 1984, Kellard *et al.* 1985).

The synthesis of methyl ketones from short chain fatty acids one carbon atom longer has been studied by many groups and is reviewed by Forney & Markovetz (1971). It follows the normal β-oxidation pathway for fatty acid oxidation up to the production of β-ketoacyl CoA, which then may be deacylated and decarboxylated to form the methyl ketone or undergo further β-oxidation. When oxygen is limiting, the methyl ketones may act as terminal hydrogen acceptors to produce secondary alcohols. Many volatile constituents have been isolated and identified from fungal metabolism (Seifert & King 1982). Terms such as musty, earthy, or mouldy are often used to describe the characteristic odours of fungal growth, particularly of *Penicillium* (Raper & Thom 1949). Oct-1-ene-3-ol and cis-2-octen-1-ol have been shown to be major components of the musty smell (Karminski *et al.* 1972, 1974, Halin *et al.* 1975).

Table 5. *Volatile compounds produced by fermentation of coconut for 30 days at ambient temperature and a^w 0.76 (mg/g oil)*

	amstelodami	Eurotium chevalieri	herbariorum	Penicillium citrinum
Pentan-2-one	2.51	trace	8.39	0.39
Pentan-2-ol	7.13	2.80	17.04	1.79
Hexan-2-one	0.52	N.D.	17.45	0.23
Hexan-2-ol	N.D.	N.D.	22.65	trace
Heptan-2-one	64.27	10.42	84.91	28.29
Heptan-2-ol	46.27	28.80	31.61	15.02
Octan-2-one	2.51	0.32	38.77	0.30
Octan-2-ol	N.D.	N.D.	2.88	0.35
Nonan-2-one	17.06	5.15	61.19	5.49
Nonan-2-ol	2.07	2.37	0.69	0.89
Undecan-2-one	8.97	8.92	35.80	5.91
Total	151	59	321	59

A major spoilage phenomenon in oils and oilseeds is the accumulation of free fatty acids. The specifications for desiccated coconut require that the free fatty acids should be less than 0.1% by weight (Sri Lankan specifications). The free fatty acids increase markedly in rancid desiccated coconut (Greenway 1985), e.g. 180 mg/g compared to 1 mg/g in oil, most being dodecanoic (107 mg/g) and tetradecanoic acids (46 mg/g). These short-chain fatty acids, in addition to the ketones and secondary alcohols, contribute to the off-odour.

IX. Conclusion

The concept of a well-defined microbial association forming during spoilage (Westerdijk 1949) holds for coconut. If storage conditions change, different organisms grow. Bacterial fermentations (producing butanediol) from carbohydrates occur at relatively high water activity and mould induced hydrolysis and oxidation of fats may lead to rancidity at lower activities.

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References

Allen, R.R. (1965) Volatile flavour constituents in coconut oil. *Chemistry and Industry*, 1565.

Bergey (1974) *Bergey's Manual of Determinative Bacteriology*. Baltimore; Williams & Wilkins.

Biffen, R.H. (1899) A fat destroying fungus. *Annals of Botany* **13**, 363-376.

CAC Recommended Codes of Practice (1971) *Recommended international codes of hygienic practice for desiccated coconut*, S-14.

Child, R. (1941) The packing and keeping qualities of desiccated coconut. *New Guinea Agricultural Gazette* **7**, 215-220.

Christensen, C.M. (1965) *The Molds and Man*. Minneapolis; University of Minnesota.

Eggins, H.O.W.; Coursey, D.G. (1968) The industrial significance of the biodeterioration of oilseeds. *International Biodeterioration Bulletin* **4**, 29-38.

Forney, F.W.; Markovetz, A.J. (1971) The biology of methyl ketones. *Journal of Lipid Research* **12**, 383-395.

Gould, G.W.; Brown, M.H.; Fletcher, B.C. (1983) Mechanisms of action of food preservation procedures. In *Food Microbiology Advances and Prospects* (T.A. Roberts; F.A. Skinner, eds), 67-82. London; Academic Press.

Greenway, I. (1985) The free fatty acid content of rancid and non-rancid desiccated coconut. *Applied Science Project*, Sheffield City Polytechnic.

Halim, A.F.; Narcisco, J.A.; Collins, R.P. (1975) Odorous constituents of *Penicillium decumbens*. *Mycologia* **67**, 1158-1165.

Kajs, T.M.; Hagenmaier, R.; Vanderzant, C.; Mattil, K.F. (1976) Microbiological evaluation of coconut and coconut products. *Journal of Food Science* **41**, 352-356.

Kaminski, E.; Libbey, L.M.; Stawicki, S.; Wasowicz, E. (1972) Identification of the predominant volatile compounds produced by *Aspergillus flavus*. *Applied Microbiology* **24**, 721-726.

Kaminski, E.; Stawicki, S.; Wasowicz, E. (1974) Volatile flavour compounds produced by molds of *Aspergillus, Penicillium* and fungi imperfecti. *Applied Microbiology* **27**, 1001-1004.

Kellard, B.; Busfield, D.M.; Kinderlerer, J.L. (1985) Volatile off-flavour compounds in desiccated coconut. *Journal of the Science of Food and Agriculture* **36**, 415-420.

Kinderlerer, J.L. (1984a) Spoilage in desiccated coconut resulting from growth of xerophilic fungi. *Food Microbiology* **1**, 3-28.

Kinderlerer, J.L. (1984b) Fungi in desiccated coconut. *Food Microbiology* **1**, 205-207.

Kinderlerer, J.L.; Clark, R.A. (1986) Microbiological quality of desiccated coconut. *Journal of Hygiene, Cambridge* **96**, 19-26.

Kinderlerer, J.L.; Kellard, B. (1984) Ketonic rancidity in coconut due to xerophilic fungi. *Phytochemistry* **23**, 2847-2849.

Kinsella, J.E.; Hwang, D.H. (1976) Enzymes of *Penicillium roquefortii* involved in the biosynthesis of cheese flavour. In *Critical Reviews in Food Sciences* (E.T. Furia, ed), 191-228.

Lin, F.M.; Wilkens, W.F. (1970) Volatile flavour components of coconut meat. *Journal of Food Science* **35**, 538-539.

Mossel, D.A.A.; Ingram, M. (1955) The physiology of microbial spoilage of foods. *Journal of Applied Bacteriology* **18**, 232-268.

Raper, K.B.; Thom, C. (1949) *A Manual of the Penicillia*. Baltimore, Williams & Wilkins.

Roberts, D.; Watson, G.N.; Gilbert, R.J. (1982) Contamination of food plants and plant products with bacteria of public health significance. In *Bacteria and Plants* (M.E. Rhodes-Roberts; F.A. Skinner, eds), 169-191. London; Academic Press.

Semple, A.B.; Graham, A.J.; Dutton, E.M. (1961) A review of the sampling of imported desiccated coconut. *The Medical Officer*, 59-60.

Seifert, R.M.; King, A.D. (1982) Identification of some volatile constituents of *Aspergillus clavatus*. *Journal of Agriculture and Food Chemistry* **30**, 786-790.

Speckman, R.A.; Collins, E.B. (1981) Preparation of radio-active diacetyl, acetoin and 2,3-butylene glycol. *Analytical Biochemitsry* **118**, 405-409.

Starkle, M. (1924) Die Methylketone im oxydativen Abbau einiger Triglyceride (bzw Fettsäuren) durch Schimmelpilze unter Berücksichtigung der besonderen Ranzidität des Kokosfettes. *Biochemische Zeitschrift* **151**, 371-415.

Stokoe, W.N. (1928) The rancidity of coconut oil produced by mould action. *Biochemical Journal* **22**, 82-93.

Thimann, K.V. (1967) *The Life of Bacteria*. New York; Macmillan.

Westerdijk, J. (1949) The concept "Association" in mycology. *Antonie van Leeuwenhoek* **15**, 187-189.

Young, F.V.K. (1983) Palm kernel and coconut oils: analytical characteristics, process technology and uses. *Journal of the American Oil Chemists Society* **60**, 374-379.

Spoilage and Mycotoxins of Cereals and other Stored Products.
Edited by B. Flannigan

MYCOTOXINS IN CEREALS AND OTHER STORED PRODUCTS

Interaction between toxic and non-toxic fungi in gamma irradiation sterilised cereals

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I. Summary

In the natural environment mycotoxins are formed in living cereal seeds in competition with the normal seed microflora. Most attempts to study these microbial interactions have made use of heat-sterilised substrates. In the present studies, a novel radiation sterilisation method has been developed which removes contaminating microorganisms without killing the seed. Studies using a microporous film containment system with irradiated seeds and toxigenic fungi either alone or in paired combination with other toxigenic fungi, non-toxigenic fungi, a yeast or a *Bacillus* sp. have been carried out in Fison Environmental Cabinets at a_w 0.90, 0.95, 0.98 and at 25°C and 16°C. Considerable variations in toxin levels were recorded.

It is well recognised that in natural exposed substrates such as cereal grains, microorganisms co-exist and will be involved in associative, competitive and/or detrimental interactions. Individual microbial species will compete for the nutrient and space available to achieve successful establishment on or in the cereal. The quantitative and qualitative composition of the cereal microflora will seldom stay static but rather will be in constant change. At any particular time there will be a characteristic microbial profile starting with the field flora and progressively changing during harvesting, transportation and storage.

II. Introduction

The ecological succession of microorganisms will be determined by the interaction of intrinsic factors (nutrient, water activity, pH, etc.) and extrinsic factors (storage temperature, water-vapour pressure, partial pressure of storage gases, etc.). During these ecological successions the microbial population will change in composition and numbers responding in part to physico-chemical changes occurring in the cereal substrate.

Many of the filamentous fungi found growing on cereals can produce mycotoxins under particular combinations of environmental conditions. The incidence of occurrence varies with type of cereal, climatic conditions and quality of treatment during harvest, transportation and storage. Of particular significance is the fact that mycotoxins are rarely uniformly distributed throughout batches of cereals, their occurrence normally being uneven and spasmodic (Smith & Moss 1985).

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There have been numerous laboratory based studies designed to unravel the complex of intrinsic and extrinsic factors regulating mycotoxin formation in cereals (see Lacey, this volume). In most of these studies the cereal substrate used to support the toxigenic mould was normally autoclaved to inactivate the resident microflora. Again most studies have involved monocultures although, more recently, attempts have been made to study dual fungal cultures. In the natural environment, when mycotoxins occur in cereals they do so normally on living grains in the presence of other naturally occurring microbial colonists. The use of heat-sterilised substrates undoubtedly will produce physico-chemical changes in the cereals making extrapolation of the results of these studies to the natural living systems unrealistic. In nature a toxigenic mould will need to penetrate the protective seed coat and, furthermore, compete with any natural microflora before achieving sufficient biomass formation to be able to produce mycotoxins.

III. Grain microflora

The surface of cereal grains before and after harvest will normally contain an extensive range of microbial species although only a limited number of species are able to enter and colonise the grain kernel. The fungi that can successfully colonise cereal grains in the field normally require high moisture levels in the kernels; maturation of the grain together with efficient drying treatment reduces the moisture level and a_w and most of these fungi will stop growing and eventually die, the rate of death depending on the water content and temperature of the grain (Christensen &Kaufmann 1974). During harvest and subsequent transportation and storage, other mould species, in particular, will settle onto the grain surface and if they are able to germinate and grow at the now lower a_w successful colonisation and possible mycotoxin production can occur.

Although filamentous fungi are the most documented of microorganisms occurring on cereals they are almost invariably present in association with bacteria, in particular psychrotrophic bacteria, actinomycetes and aerobic sporulating bacteria. Yeasts also occur in not inconsiderable numbers but do not normally constitute a spoilage problem in correctly dried grain.

IV. Intrinsic factors influencing microbial growth and interaction

Only two aspects within the broad category of intrinsic factors will be briefly considered, viz. substrate and antimicrobial factors, as they have particular significance to the present studies.

Substrate: The type of cereal substrate plays a profound role in both mould growth and toxin production. The physical nature of the seed coat can have a marked effect on mould infection, colonisation and toxin production (Chelkowski *et al.* 1981).

The hyphae of *Aspergillus flavus* can enter through cracks in the perideum and penetrate the endosperm of rice (Ilag & Juliano 1981). In contrast, more aflatoxin production occurred on covered barley cultivars than on those without hulls (Chang & Markakis 1981).

If the structure and/or physical form of the substrate has a pronounced effect on infection and colonisation, cereal composition also contribute in selecting both the range and type of fungi and other organisms able to grow on or within the seed mass, and, where relevant, the type and level of mycotoxin produced. Substrates with high carbohydrate and lipid content tend to favour aflatoxin production. The effect of different types of substrate (heat-sterilised) on aflatoxin formation was clearly demonstrated by Hesseltine *et al.* (1966) using three different strains of *A. flavus* on rice, sorghum, peanuts, maize, wheat and soybeans. Although all strains grew well on most substrates they produced widely different amounts of aflatoxin. In particular, soybeans were a poor substrate for aflatoxin sythesis.

Similarly, production of zearalenone by *Fusarium roseum* was greater on polished rice than on maize and wheat, with much less on oats and barley and none in soybeans or peas (Eugenio *et al.* 1970). Conversely, others have found zearalenone on soybeans and a greatly enhanced production of the trichothecene HT-2 in soybean meal compared to rice culture (Richardson *et al.* 1984).

Antimicrobial compounds: Inherent in any concept of microbial interactions in cereals will be the possible role of chemical compounds released into the substrate by the resident microorganisms in establishing some form of biochemical communication between species. In particular, the filamentous fungi are prodigious producers of secondary metabolites, many of which are known to be mycotoxins. It has been suggested that mycotoxins released by toxigenic fungi might function in nature as antimicrobial compounds, thus ensuring selective advantage to the colonising toxigenic fungus. However, most mycotoxins, for example aflatoxin, have little antimicrobial activity, while others which are obviously antimicrobial have not been demonstrated in nature in quantities likely to provide a competitive advantage to the producer (Lillehoj 1980). Ciegler (1983) has hypothesised that in an ecological context naturally produced mycotoxins may have a multiplicity of functions, e.g. as antibiotics, as chemical signalling agents, as mutagenic agents, etc. However, the role of secondary metabolites *per se* is still unclear (Hutter 1982). In particular, secondary metabolism is linked with

growth and is probably part of the normal maturation process of the producer organism and may be a form of physiological adaptation.

V. Extrinsic factors affecting microbial growth and interaction

Of particular consequence in selecting seed microflora will be environmental factors such as storage temperatures, water vapour pressure and partial pressure of the atmospheric gases. The potential for microbial growth will be a combination of instrinsic and extrinsic environmental factors coupled with the genetic properties of the microorganisms; thus in a particular environment an organism grows in a characteristic manner and at a characteristic rate. While organisms remain metabolically active they will continue to interact. Thus, dominance within a microflora may not be permanent but dynamic. Interactions can be synergistic or antagonistic depending on whether the effect is growth enhancing or inhibitory.

VI. Mycotoxin production in mixed microbial systems

Mycotoxin production has mostly been studied in monocultures on rich organic media under controlled environmental conditions. There have been relatively few attempts to study growth of toxigenic fungi and mycotoxin formation in mixed cultures in either synthetic media or natural substrates or in fluctuating environmental conditions.

In early studies with peanuts (Ashworth *et al.* 1965) it was shown that development of *A. flavus* and production of aflatoxin was regulated by competition with other fungi that could invade the peanut kernels, such as *A. niger* and *Rhizoctonia solani*. However, to achieve such reduction of aflatoxin production it was necessary to prior-inoculate with the antagonistic organisms. In a similar way, simultaneous inoculation of rice with *A. chevalieri* or *A. candidus* decreased aflatoxin production by *A. parasiticus* compared to simple culture of *A. parasiticus* (Boller & Schroeder 1973, 1974). They suggested that the toxin decrease resulted from the inhibition of *A. parasiticus* by the dominant *A. chevalieri* and *A. candidus* rather than by metabolism of the toxin. Conversely, Alderman *et al.* (1973) reported an increase of aflatoxin in mixed culture of *A. flavus* with *P. italicum* or *Lactobacillus plantarum*.

No aflatoxin was formed when *A. flavus* was paired with either *A. niger* or *Trichoderma viride* on autoclaved maize (Wicklow *et al.* 1980). Aflatoxin production was detected, however, when *A. flavus* was paired with *Candida guilliermondii*. The authors considered that the competitor species influenced the biosynthetic pathways of aflatoxin production.

Several groups have reported substantial to total inhibition of aflatoxin production when autoclaved rice or maize was simultaneously inoculated with either *A. flavus* or *A. parasiticus* and competing *A. niger* (e.g. Tsubouchi *et al.* 1981). The reduction in aflatoxin production in the presence of the competing *A. niger* appears to involve factors other than competitive exclusion.

This may involve enzymatic degradation of the aflatoxin, inhibition of toxin formation by the low pH generated by *A. niger* or by the production of inhibitory metabolites (Boller & Schroeder 1973, Horn & Wicklow 1983).

For our present studies on mycotoxin formation in cereals an experimental protocol was required that would ensure initially microorganism-free living seeds and a containment system that would facilitate environmental control of water activity, temperature and aeration together with aseptic operation. Such a system has now been developed and used to study the interaction between toxigenic fungi and other microorganisms, in particular filamentous fungi, yeasts and bacteria (Cuero *et al.* 1985).

VII. Seed sterilisation by irradiation

Sterilisation of seeds in the laboratory is commonly carried out by heat or chemical treatment. Surface sterilisation of the seeds with sodium hypochorite although efficient for surface containment still leaves internal living microbial colonies (Flannigan 1977). Autoclaving ensures a microbe-free substrate but the seed is killed and drastically altered in a structural and chemical manner.

Gamma-irradiation is an alternative method for seed sterilisation. The susceptibility of various microorganisms to radiation has been well studied (Kiss & Farkes 1977) and its effectiveness for preserving foodstuffs from microbial growth and insect infestation has been well demonstrated (IAEA-FAO 1970, 1978). Microorganisms show a wide range of susceptibilities to irradiation (Ito *et al.* 1973, Mohyuddin & Skoropad 1973). Yeasts and bacterial spores are distinctly more resistant than moulds.

For the present study, maize seeds were equilibrated to 22% moisture content at 4°C for two weeks prior to exposure to various irradiation dosages (Cuero *et al.* 1986). A specific radiation dosage of 1200 Krad eliminated all seed-borne microorganisms, while still retaining the ability of the seed to

germinate. Microorganisms varied in their sensitivity to gamma irradiation. In particular, phycomycete species, *Penicillium* spp. and *Aspergillus* spp. were much more sensitive to irradiation than *Fusarium* spp. *Saccharomyces* spp. and *Bacillus* spp.

The aspergilli and penicilli were eliminated by 120 Krad. The Phycomycetes occurred up to 120 Krad but were eliminated at 600 Krad. Yeast and *Fusarium* spp. were particularly resistant to irradiation and were only eliminated by treatment at 1200 Krad.

A comparison of sodium hypochlorite and irradiation treatment of maize for the removal of microorganisms is shown in Table 1. Table 1 also shows the effect of these treatments on seed germination. Sodium hypochlorite markedly reduced germination without completely eradicating microbial contamination. In contrast, low dosages of irradation appeared to stimulate germination of the maize seeds.

VIII. Containment system

The experimental system comprised Fison Environmental Cabinets with temperature and humidity controls in which the containment bags containing the irradiated cereal and inoculum were placed (Cuero *et al.* 1985). The bags were made from Valmic microporous film, a specially formulated opaque white polypropylene material with micropores less than 0.4 μm. This bag material was initially developed for mushroom spawn production and is now extensively used by the French spawn-maker Somycel and is the subject by them of a patent. The microporous bags can be autoclaved and used repeatedly. In the present system the size of the bags was 13 x 23 cm and they were sealed by impulse heating. The most important features of the bag is the Valmic microporous film which allows efficient air (c. 25 $cm^3/cm^2/min$ at 1 atm) and moisture vapour transmission (c.500 $g/m^2/24$ h at 23°C and 50% RH) with no cross movement of fungal spores. Throughout the experiments (1-2 weeks) internal RH and temperature values, as determined by an electronic dew-point meter (Protimeter DP 680), were relatively constant, reaching their steady values not significantly different from the external conditions in approx. 2 hours.

Table 1. *The examination of maize seeds at 15 and 22% moisture content after sodium hypochlorite or gamma irradiation treatment*

	Germination (%)*	
Treatment	15% MC	22% MC
Sodium Hypochlorite	59	55
Gamma Irradiation (Krad)		
from 30 to 360	95	92
420	90	90
480	95	95
540	86	83
600	86	82
1200	75	72
1800	0	0
Untreated	79	73

*Results are derived from three experiments, each experiment with a total of 100 seeds (2 seeds/wet filter paper), after 5 days at 25°C and 90% RH.

IX. Toxin production on irradiated maize and rice seeds contained in the microporous bags

For the following experiments maize or rice seeds were sterilised by gamma-irradiation at 1200 Krad and contained aseptically in the microporous bags in the Fison Cabinets set at the appropriate RH and temperature. A_w levels of 0.98, 0.95 and 0.90 were used throughout and two temperatures, viz. 25° and 16°C, were selected.

Toxigenic fungi were grown separately in the microporous bags at the three a_w values and both temperatures and the bags monitored regularly for growth morphology. At the end of the experimental period the contents of the bags were removed and analysed for the respective toxins. Three toxigenic fungi were primarily used in these studies, viz. *Aspergillus flavus, Penicillium viridicatum* and *Fusarium graminearum*, producing respectively aflatoxin, ochratoxin A and zearalenone.

In separate studies each toxigenic fungus was paired separately with each of the other toxigenic fungi or non-toxigenic fungi, viz. *A. niger* and *A. oryzae*. Similar pair-inoculations were carried out with a *Saccharomyces* sp. and a *Bacillus* sp. isolated from the maize seeds. Growth of the mixed cultures was monitored visually and toxin analysis carried out at the completion of the experiment.

In the context of this presentation only the growth of *A. flavus* in single and mixed cultures on two substrates will be noted.

In single culture, aflatoxin production increased with increasing a_w values on maize while on rice there was no toxin production at any of the a_w values. Production of aflatoxin was highest at 25°C and a_w 0.98. There was a close positive correlation between the amounts of toxin produced and water activity at both temperatures, with high correlation coefficient values of 0.929 at 25°C and 0.869 at 16°C.

In paired cultivation with *A. oryzae* total aflatoxin on maize maintained the same approximate values at a_w levels of 0.98 and 0.95 at 25°C when compared to single cultures of *A. flavus*. Aflatoxin levels were noticeably lower at 0.90 a_w at 25°C and at all a $_w$ values at 16°C. Again there was no toxin production on rice. There was direct correlation on maize between a_w at both temperatures and aflatoxin production. The highest coefficient correlation value was 0.90 under all a_w values at 16°C and the lowest value was 0.34 under all a_w values at 25°C.

In paired cultivation with either *Saccharomyces sp. or Bacillus* sp. aflatoxin production occurred in both substrates. In maize levels of aflatoxin were much higher in both mixed cultures than in the monocultures.

References

Alderman, G.G.; Emeh, C.O.; Marth, E.H. (1973). Aflatoxin and rubratoxin production by *Aspergillus parasiticus* and *Penicillium rubrum* when grown independently, associatively or with *P. italicum* or *Lactobacillus plantarum*. *Zeitschrift für Lebensmittel-untersuchung und -forschung* **153**, 305-311.

Ashworth, L.J.; Schroeder, H.W.; Langley, B.C. (1965). Aflatoxins : environmental factors governing occurrence in Spanish peanuts. *Science* **148**, 1228-1229.

Boller, R.A.; Schroeder, H.W. (1974). Influence of *Aspergillus candidus* on production of aflatoxin in rice by *Aspergillus parasiticus*. *Phytopathology* **64**, 121-123.

Boller, R.A.; Schroeder, H.W. (1973). Influence of *Aspergillus chevalieri* on production of aflatoxin in rice by *Aspergillus parasiticus*. *Phytopathology* **63**, 1507-1510.

Chang, H.G.; Markakis, P. (1981). Effect of gamma irradiation on aflatoxin production in barley. *Journal of the Science of Food and Agriculture* **33**, 559-564.

Chelkowski, J.; Dopierala, G.; Godlewska, B.; Radomyska, W.; Szebiotko, K. (1981). Mycotoxins in cereal grain. Part III. Production of ochratoxin A in different varieties of wheat, rye and barley. *Die Nährung* **25**, 625-629.

Christensen, C.M.; Kaufmann, H.H. (1974). Microflora. In *Storage of cereal grains and their products* (C.M. Christensen, ed.), 158-192. St Paul; American Association of Cereal Chemists.

Ciegler, A. (1983). Do mycotoxins function in ecological processes? *Journal of Food Protection* **5**, 23-29.

Cuero, R.G.; Smith, J.E.; Lacey, J. (1985). A novel containment system for laboratory scale solid particulate fermentations. *Biotechnology Letters* **7**, 463-466.

Cuero, R.G.; Smith, J.E.; Lacey, J. (1986). Influence of gamma-irradiation and sodium hypochlorite on the microflora and germination of maize seeds. *Journal of Food Microbiology* (in press).

Eugenio, C.P.; Christensen, C.M.; Mirocha, C.J. (1970). Factors affecting production of mycotoxin F-2 by *Fusarium roseum*. *Phytopathology* **60**, 1055-1057.

Flannigan, B. (1977). Enumeration of fungi and assay for ability to degrade structural and storage components of grain. In *Biodeterioration Investigation Techniques*. (A.H. Walters, ed.), 185-199. London; Applied Science.

Hesseltine, C.W.; Shotwell, O.L.; Ellis, J.J.; Stubblefield, J. (1966). Aflatoxin formation by *Aspergillus flavus*. *Bacteriological Reviews* **30**, 795-805.

Horn, B.W.; Wicklow, D.T. (1983). Factors influencing the inhibition of aflatoxin production in corn by *Aspergillus niger*. *Canadian Journal of Microbiology* **29**, 1087-1091.

Hutter, R. (1982). Design of culture media capable of provoking wide gene expression. In *Bioactive Microbial Products : Search and Discovery*. (J.D. Bu'Lock; L.J. Nisbet; D.J. Winstanley, eds), 37-50. London; Academic Press.

IAEA-FAO. (1970). *Training manual on food irradiation technology and techniques*. Technical Reports Series No. 114. Vienna; IAEA.

IAEA-FAO. (1978). *Food preservation by irradiation,* Vol. I. Proceedings of a Symposium, Wageningen, 21-25 November 1977. Vienna; IAEA.

Ilag, L.L.; Juliano, B.O. (1981). Colonization and aflatoxin formation by *Aspergillus* spp. on brown rices differing in endosperm properties. *Journal of Science & Food Agriculture* **33**, 97-102.

Ito, H.; Iizuka, H.; Sato, T. (1973). Identification of osmophilic *Aspergillus* isolated from rice and their radiosensitivity. *Agricultural and Biological Chemistry* **37**, 789-798.

Kiss, I.; Farkas, J. (1977). The storage of wheat and corn of high moisture content as effected by ionizing radiation. *Acta Alimentaria* **6**, 193-214.

Lillehoj, E.B. (1980). Secondary metabolites as chemical signals between species in an ecological niche. In *Proceedings, Sixth International Fermentation Symposium, London, Ontario.*

Moyhuddin, M.; Skoropad, P. (1973). Effect of ^{60}Co gamma irradiation on the survival of some samples of each of three different grades of wheat. *Canadian Journal of Botany* **48**, 217-219.

Richardson, K.E.; Hagler, W.M.; Hanney, C.; Hamilton, P.B. (1984). Zearalenone and trichothecene production in soyabean by toxigenic *Fusaria*. *Journal of Food Protection* **48**, 240-243.

Smith, J.E.; Moss, M.O. (1985). *Mycotoxins : formation, analysis and significance*. Chichester; John Wiley.

Tsubouchi, H.; Yamamoto, K.; Hisada, K.; Sakabe, Y.; Tsuchihira, K. (1981). Degradation of aflatoxin B_1 by *Aspergillus niger*. *Proceedings, Japanese Association of Mycotoxicology* **12**, 33-35.

Wicklow, D.T.; Hesseltine, C.W.; Shotwell, O.L.; Adams, G.L. (1980). Interference competition and aflatoxin levels in corn. *Phytopathology* **70**, 761-764.

Spoilage and Mycotoxins of Cereals and other Stored Products.
Edited by B. Flannigan

Moulds and Mycotoxins in Cereal and Other Components of Compound Animal Feeds

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I. Summary

Mould spoilage of cereals and other components of compound animal feeding stuffs may lead to the production of mycotoxins. The commonly reported occurrence of mycotoxins in stored products is indicative that mould spoilage is a widespread problem before and/or after harvest of many cereals and oil-seed crops throughout the world. Commodities differ in their pre-disposition to fungal damage which is influenced by the climatic conditions under which they are grown and stored. The animal feeding stuffs industry in the UK uses a mixture of home-grown and imported cereals, cereal by-products, oil-seeds and animal proteins in compound feeding stuffs. Economic and nutritional factors play a major part in influencing the composition of compound feeding stuffs. Until recently less attention was given to the presence of mycotoxins in feed ingredients and the possible consequences that they could have on animal production. Aflatoxin B_1 is the only mycotoxin covered by the Feeding Stuffs Regulations (1982) which define the maximum content permitted in various feeding stuffs. However, legislative controls alone cannot ensure that feeding stuffs do not contain harmful levels of mycotoxins since poor storage on farms can lead to mould spoilage and mycotoxin contamination.

II. Introduction

The outbreak of 'Turkey X Disease' in England during 1960 and the subsequent discovery of aflatoxin generated an international interest in mycotoxins which has gathered momentum in the past 10 years. The development of more sensitive analytical methods has made it possible to identify an increasing number of naturally occurring compounds produced by moulds on many agricultural commodities. These discoveries have led to numerous toxicological studies and given rise to concern that mycotoxin contamination of animal feeding stuffs may impair productivity or cause disease. In recognising this possibility the Agricultural Development and Advisory Service (ADAS) of the Ministry of Agriculture, Fisheries and Food (MAFF) has carried out mycotoxin analysis of animal feeding stuffs and their components over the past 10 years. The findings have revealed that mycotoxins occur commonly in cereals and animal feeding stuffs submitted for examination in England and Wales. However, the levels at which they have been detected have rarely been considered sufficiently high to have had any adverse effects on animal performance.

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III. The Formulation of Compound Animal Feeding Stuffs

Present day methods of animal production are largely dependent on compound feeding stuffs to supply the nutritional requirements of livestock. Pig and poultry feeding stuffs are usually formulated as complete feeds whereas ruminant compounds are formulated to contain energy, proteins, vitamins and minerals in a concentrated form to complement forage, i.e. grass, hay and silage. The use of compound feeding stuffs makes for an easy and reliable way of ensuring that livestock receive the correct balance of nutrients to achieve optimum performance. The annual production of compounds in the UK during the past 10 years has been in the region of 10 to 12 million tonnes. In addition, there has been an increased production of home-mixed feeds on many farms using bought-in raw materials, e.g. soya, palm kernels and maize gluten for compounding with cereals grown on the farm.

The formulation of feeding stuffs is determined by nutritional requirements and economic factors. Animal feeding stuffs manufacturers can choose from a wide variety of raw materials for compounding together in the appropriate proportions to achieve a particular nutritional specification. In practice, feeds are formulated on a least cost basis within certain fixed compositional criteria, i.e. minimum and maximum limits are set for the inclusion level of a particular commodity. This means that formulations vary from year to year in response to fluctuations in prices on the international commodity markets, the value of sterling, import quotas and changes in legislation. The possible presence of mycotoxins may be of lesser concern and may not be discovered until after a particular consignment of a commodity becomes implicated in causing a feed related problem.

IV. Imported Commodities

The trade in manioc (tapioca) and groundnut illustrates how economic factors and legislation can influence the composition of feeding stuffs. In 1981/82, manioc imported from Thailand was very competitively priced with EEC cereals. As a result, the import of manioc into the UK increased from 26,300 tonnes in 1979/80 (August-July) to 816,000 tonnes in 1981/82, when it was used as a cereal replacement, because of its high starch content (metabolisable energy 12.7 MJ/kg), in combination with soya. It was fortuitous that manioc was not a significant source of mycotoxins although some consignments were found to contain up to 0.020 mg/kg of aflatoxin B_1. However, the imposition of import quotas of manioc by the EEC reduced imports into the UK in 1984/85 to about 10% of the level imported in 1981/82.

Groundnut was identified as the source of aflatoxin in the outbreak of Turkey X Disease but continued to be used widely by the feed industry in compound dairy feeds until 1981 when a temporary ban was placed on its import into the UK. This followed the discovery of aflatoxin M_1 in milk and milk products (HMSO, 1980) which implicated groundnut as the principal source of aflatoxin B_1 in the feed. This ban was later relaxed in 1982 to permit the import of groundnut provided that the level of aflatoxin B_1 did not exceed 0.050 mg/kg. This had the effect of reducing the import of groundnut into the UK from 168,000 tonnes in 1979/80 (August to July) to a mere 600 tonnes in 1982/83. Most of the other oil-seed products which are commonly incorporated in feeding stuffs are a potential source of aflatoxin although they generally contain much lower levels than are found in groundnut. The occurrence of aflatoxin in palm kernels is the subject of another paper (see Cornelius and Maduagwu in this volume).

Other oil-seed products found to be contaminated with aflatoxin include cottonseed (Whitten 1969, Price *et al.* 1985), soybeans (Shotwell *et al.* 1969), copra (A.E. Buckle, unpublished) and sunflower (A.E. Buckle, unpublished).

Cereals constitute a major component of compound feeding stuffs (Table 1). They include both home-grown cereals (wheat and barley) and imported maize and its by-products. Maize is susceptible to mould damage before and after harvest. In the field it is subject to invasion by *Fusarium* species, which can result in the production of zearalenone (Sutton *et al.* 1980) and deoxynivalenol (Vesonder *et al.* 1978). Maize is also vulnerable to invasion by *Aspergillus flavus*, particularly in times of drought when it is more prone to insect damage. Colonisation by *A. flavus* can lead to the production of aflatoxin before harvesting, and subsequently in store if the grain is not dried to less than 14% moisture content. Many surveys of maize in the USA have revealed aflatoxin contamination to be a common occurrence especially in low grade maize (Shotwell 1977), which may be used for alcohol production. The fermentation process has no effect on aflatoxin and zearalenone which become concentrated in the solids and soluble fraction after distillation of the ethanol (Hesseltine 1984). The solid by-product known as distillers maize is used in animal feeding stuffs. It has been analysed by MAFF to reveal deoxynivalenol at 1.0 mg/kg and traces (less than 0.020 mg/kg) of aflatoxin.

Table 1. *Proportions of raw materials used in the manufacture of compound feeding stuffs in the UK*

	1981 %	1984 %
Wheat	23.2	29.3
Wheat by-products	8.5	8.4
Barley	15.9	9.0
Maize	8.1	2.8
Oilseeds	15.6	13.8
Animal by-products	4.5	4.9
Oils and fats	1.7	1.7
Molasses	3.9	4.3
Others	21.5	25.8

V. UK Produced Cereals

Home-grown cereals rarely undergo sufficient spoilage by *Fusarium* species before harvest to result in significant amounts of *Fusarium* mycotoxins being produced. A survey carried out by MAFF (Gilbert *et al.* 1983) on 87 samples of both feeding and malting barleys grown in England and Scotland showed levels of less than 0.02 mg/kg of deoxynivalenol in 90% of the samples. The level exceeded 0.10 mg/kg in only 2.3% of the samples. In a survey of the occurrence of seven trichothecenes in UK home grown wheat carried out by the Flour Milling and Baking Research Association (Osborne & Willis 1984) 199 samples were examined. Deoxynivalenol was the only compound detected and was found to occur in 16% of the samples at levels ranging from 0.02 to 0.40 mg/kg. These findings contrast with reports from North America of much higher levels of deoxynivalenol in wheat. Trenholm *et al.* (1981) reported an average level of 0.93 mg/kg of deoxynivalenol in 28 samples of feed grade wheat grown in Ontario. Hagler *et al.* (1984) reported an average level of 1.78 mg/kg of deoxynivalenol in 33 samples of scabby wheat grown in Nebraska. In addition, some of these wheat samples were also found to contain zearalenone and aflatoxin B_1 in various combinations. T-2 toxin was not found in any of the samples.

The most commonly found mycotoxins in home-grown wheat and barley appear to be ochratoxin A and citrinin. They have been found in the course of advisory and survey work by ADAS on grain which has gone mouldy in store. These mycotoxins are produced by *Penicillium viridicatum* which commonly colonises grain that has not been properly dried to below 15% moisture content. A survey carried out by ADAS microbiologists of the 1979 harvest of barley sampled after 10 months' storage on 93 farms in England and Wales showed that about 16% of the samples contained one or more mycotoxins (Table 2).

Table 2. *Occurrence of mycotoxins in barley stored on farms in England and Wales*

No. of farms surveyed	No. of samples examined	No of samples positive OA	C	OA+C	S
93	108	6	3	7	1

OA = Ochratoxin A
S = Sterigmatocystin
C = Citrinin

Ochratoxin A and citrinin were often found together. Sterigmatocystin was found in one sample from which *Aspergillus versicolor* was the predominant mould isolated.

These findings have been corroborated by more recent results from analysing home-grown grain submitted to ADAS by the feed industry for mycotoxin analysis in 1985. Out of 140 samples of wheat and barley 39, 35 and 6 were respectively found to contain ochratoxin A, citrinin and sterigmatocystin, sometimes occurring in combination. It is important to note that these analyses were requested because the grain was visibly of questionable quality and was therefore unrepresentative of the grain used in the manufacture of animal feeding stuffs. Nevertheless these findings do reveal that much grain is not being adequately dried and stored on many farms in England and Wales and is consequently subject to mould spoilage and mycotoxin contamination.

VI. Mycotoxins in Compound Animal Feeding Stuffs

In view of the common occurrence of mycotoxins in many of the components used in compound animal feeding stuffs it is predictable that these will be found to contain a variety of mycotoxins. The mycotoxins have been found in compound feeding stuffs in Canada (Prior 1981), Australia (Connole *et al.* 1981) and England and Wales (Buckle 1983). Since 1976, over 1400 samples of compound animal feeding stuffs have been examined by ADAS for mycotoxins using the method of Patterson & Roberts (1979). Those mycotoxins found using this method have included aflatoxin B_1, ochratoxin A, citrinin and zearalenone. T-2 toxin and diacetoxyscirpenol have never been found. Prior to the restrictions put on the import of groundnut, aflatoxin B_1 was often found in compound feeding stuffs. For example, in 1980 it was detected in about 30% of compound dairy feeds though at a lower incidence in pig and poultry feeds (Table 3).

Table 3. *Aflatoxin B_1 in compound feeding stuffs examined in 1980*

		Aflatoxin B_1 (μg/kg)				
Feed	+/total	<5	6–20	21–50	51–100	>100
Dairy	62/206	17	24	19	–	2
Beef	4/10	2	1	–	1	–
Pig	7/70	2	2	–	1	2
Poultry	8/127	1	6	1	–	–

During the same period aflatoxin was also detected in many of the commodities used in compound feeding stuffs (Table 4).

Groundnut was the most important source of aflatoxin; it was often present at levels exceeding 1.0 mg/kg. The restrictions put on its import have brought about a marked reduction in the incidence

Table 4. *Aflatoxin B_1 in straights examined in 1980*

		Aflatoxin B_1 (μg/kg)				
Straight	+/total	<20	21–1 0	51–100	101–1000	>1000
Groundnut	22/37	1	1	3	9	8
Palm kernels	6/7	–	5	1	–	–
Cotton seed	5/5	3	–	1	1	–
Maize	4/30	3	–	–	1	–
Copra	2/5	2	–	–	–	–
Rice bran	4/7	4	–	–	–	–

and level of aflatoxin B_1 found in compound feeding stuffs in recent years. Furthermore, the Feeding Stuffs Regulations (1982, No. 1143) were amended (Table 5) to halve the maximum allowable content of aflatoxin B_1 to 0.010 mg/kg in complementary feeding stuffs for dairy cattle. The Regulations do not apply to any other mycotoxins.

Legislation alone cannot ensure that a feeding stuff will not contain harmful levels of mycotoxins when eaten by livestock. Once it has been delivered to a farm it may undergo mould spoilage which is liable to result in the formation of mycotoxins. Feed silos are usually located next to livestock buildings and are exposed to the sun. In poorly insulated silos this can give rise to localised heating and the consequent migration of moisture and formation of condensation on the cooler north side of the silo. This problem will be exacerbated if a batch of feed is delivered still warm from the pelleting process. The build up of moisture on the cooler side of a feed silo may raise the water activity sufficiently to initiate growth by the more xerophilic moulds belonging to the *Aspergillus glaucus* group. A succession of mould species may develop leading to the establishment of *Penicillium viridicatum* and the ultimate production of ochratoxin A and/or citrinin in extreme cases. Mycotoxins are not necessarily produced by superficial mould spoilage, e.g. by *Aspergillus glaucus*, yet aflatoxin B_1 could be present due to the inclusion of a contaminated ingredient. In advisory work it may be of crucial importance to discern where mycotoxin contamination has arisen. For example, a pelleted pig feed may contain maize gluten contaminated with deoxynivalenol. If it is stored under damp conditions it could become colonised by aspergilli and penicillia. Should there be sufficient deoxynivalenol present to cause feed rejection or reduced intake the farmer might complain to the feed compounder who could respond that the deoxynivalenol had been formed because of spoilage of the feed at the farm. The absence of *Fusarium* species on the feed could be used to dismiss the possibility that deoxynivalenol had been produced at the farm and the presence of aspergilli and

Table 5. *Feeding Stuffs Regulations*

Feeding stuff	Aflatoxin B_1 Maximum content (mg/kg)
Straight feeding stuffs	0.05
Complete feeding stuffs for cattle, sheep and goats (except dairy cattle, calves, lambs and kids)	0.05
Complete feeding stuffs for pigs and poultry (except piglets and chicks)	0.02
Complementary feeding stuffs for dairy cattle	0.01
Other complete feeding stuffs	0.01

penicillia would be of little significance. However if ochratoxin A was present, which could give rise to excessive thirst and increased urination in the pigs, it would not be possible to ascertain if it was already present in the cereal constituents of the pellets or whether it had been produced by *Penicillium viridicatum* spoilage at the farm.

VII. The Significance of Moulds and Mycotoxins in Animal Feeding Stuffs

The occurrence of moulds and mycotoxins in animal feeding stuffs has received much attention in recent years but it should be realised that the very low levels at which identifiable mycotoxins have usually been found have not been shown to cause any harmful effects on livestock or significant production losses. There is understandable concern that mycotoxin residues or their metabolised derivatives in meat and animal products might contaminate human food though at the extremely low levels at which these have been detected, e.g. aflatoxin M_1 in milk and milk products (HMSO, 1980), there is no proof that they are harmful.

In view of this, it is appropriate to question whether the attention that mycotoxins have received in recent years is justified. From a scientific viewpoint they offer a very fertile area of research with the prospect of identifying many more naturally occurring potentially toxic substances in animal feeding stuffs and the food we eat. In our quest for mycotoxins it should always be borne in mind that they are indicators of fungal spoilage. Even if insufficent mycotoxins are present to cause any adverse effects it is a sign that the feed will be nutritionally inferior. Bartov *et al.* (1982) have reported that spoilage by *Aspergillus* and *Penicillium* species in maize and sorghum stored at 15% moisture content reduced the metabolisable energy content but did not result in the formation of aflatoxin B_1, ochratoxin A, sterigmatocystin, patulin or zearalenone. When the maize and sorghum were used in rations fed to chicks significant differences were observed in performance. Chicks fed the ration containing either the mould spoiled maize or sorghum had significantly lower weight gains than a control group of chicks fed rations containing the same batches of maize or sorghum which had not been allowed to mould.

In a study by Jones *et al.* (1982) of commercial broiler operations in the USA it was suggested that a very low level of aflatoxin B_1 (0.014 mg/kg), which had been formed in feed whilst in store at farms, resulted in productivity losses. The authors did not consider the possibility that these losses alternatively may have been attributable to a reduction in the nutritional value of the feed arising out of microbial activity. This concept has received scant attention in recent years though it is not original. Gussow (1907) wrote "it must be clearly understood that every fungus alike renders the plants attacked by it less nutritious, on account of their parasitic mode of life". It is a perfectly valid point of view, and if it is substantiated then new methods for the rapid detection of moulds and mycotoxins could prove to be very useful tools in predicting the nutritional quality of cereals and other components of compound animal feeding stuffs.

References

Bartov, I.; Paster, N.; Lisker, N. (1982) The nutritional value of moldy grains for broiler chicks. *Poultry Science* **61**, 2247-2254.

Buckle, A.E. (1983) The occurrence of mycotoxins in cereals and animal feedstuffs. *Veterinary Research Communications* **7**, 171-186.

Connole, M.D.; Blaney, B.J.; McEwan, T. (1981) Mycotoxins in animal feeds and toxic fungi in Queensland, 1971-80. *Australian Veterinary Journal* **57**, 314-318.

Gilbert, J.; Shepherd, M.J.; Startin, J.R. (1983) A survey of the occurrence of the trichothecene mycotoxin deoxynivalenol (vomitoxin) in UK grown barley and in imported maize by combined gas chromatography-mass spectrometry. *Journal of the Science of Food and Agriculture* **34**, 86-92.

Gussow, H.T. (1907) Injurious fodder and poisonous plants. *Journal of the Royal Agricultural Society of England* **68**, 32-63.

Hagler, W.M.; Tyczowska, K.; Hamilton, P.B. (1984) Simultaneous occurrence of dioxynivalenol, zearalenone and aflatoxin in 1982 scabby wheat from the midwestern United States. *Applied and Environmental Microbiology* **47**, 151-154.

Hesseltine, C.W. (1984) Mycotoxins and alcohol production: A review. In *Toxigenic fungi - their toxins and health hazard* (H. Kurata and Y. Ueno, eds), 153-161. Tokyo; Kodansha/Elsevier.

HMSO (1980) Survey of mycotoxins in the United Kingdom. In *Food Surveillance Paper No. 4*. London; Ministry of Agriculture; Fisheries and Food.

Jones, F.T.; Hagler, W.M.; Hamilton, P.B. (1982) Association of low levels of aflatoxin in feed with productivity losses in commercial broiler operations. *Poultry Science* **61**, 861-868.

Osborne, B.G.; Willis, K.H. (1984) Studies into the occurrence of some trichothecene mycotoxins in UK home-grown wheat and in imported wheat. *Journal of the Science of Food and Agriculture* **35**, 579-583.

Patterson, D.S.P.; Roberts, B.A. (1979) Mycotoxins in animal feedstuffs: Sensitive thin layer chromatographic detection of aflatoxin, ochratoxin A, sterigmatocystin, zearalenone and T-2 toxin. *Journal of the Association of Official Analytical Chemists* **62**, 1265-1267.

Price, R.L.; Paulson, J.H.; Lough, O.T.; Gingg, C.; Curtz, A.G. (1985) Aflatoxin conversion by dairy cattle consuming naturally-contaminated whole cottonseed. *Journal of Food Protection* **48**, 11-15.

Prior, M.G. (1981) Mycotoxins in animal feedstuffs and tissues in Western Canada 1975 to 1979. *Canadian Journal of Comparative Medicine* **45**, 116-119.

Shotwell, O.L.; Hesseltine, C.W.; Burmeister, H.R.; Kwolek, W.F.; Shannon, G.M.; Hall, H.H. (1969) Survey of cereal grains and soybeans for the presence of aflatoxin. II. Corn and soybeans. *Cereal Chemistry* **46**, 454-463.

Shotwell, O.L. (1977) Aflatoxin in corn. *Journal of the American Oil Chemists' Society* **54**, 216A-224A.

Sutton, J.C.; Baliko, W.; Funnell, H.S. (1980) Relation of weather variables to incidence of zearalenone in corn in Southern Ontario. *Canadian Journal of Plant Science* **60**, 149-155.

Trenholm, H.L.; Cochrane, W.P.; Cohen, H.; Elliot, J.I.; Farnworth, E.R.; Friend, D.W.; Hamilton, R.M.G.; Neish, G.A.; Standish, J.F. (1981) Survey of vomitoxin contamination of the 1980 white winter wheat crop in Ontario, Canada. *Journal of the American Oil Chemists' Society* **58**, 992A-994A.

Vesonder, R.F.; Ciegler, A.; Rogers, R.F.; Burbridge, K.A.; Bothast, R.J.; Jensen, A.H. (1978) Survey of 1977 crop year preharvest corn for vomitoxin. *Applied and Environmental Microbiology* **36**, 885-888.

Whitten, M.A. (1969) Screening cottonseed for aflatoxins. *Journal of the American Oil Chemists' Society* **46**, 39-40.

Spoilage and Mycotoxins of Cereals and other Stored Products.
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Fusarium Mycotoxins in Cereals and other Stored Products.

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Summary

A review emphasising recent literature is presented of *Fusarium* mycotoxins in cereals and other stored products. Zearalenone and the trichothecenes are discussed in detail and current information on compounds such as moniliformin, butenolide and fusarin C is summarised. Toxin structures, toxicity, factors affecting the formation and degradation of toxins, analytical approaches, natural occurrence, and fate in the chain are described.

I. Introduction

Fusarium moulds are frequent pathogens of a wide variety of crops, particularly cereals, and their spores may invariably be isolated in large numbers even from healthy plants. The moulds may produce an extensive range of metabolites (Fig. 1) with diverse toxic effects. One toxin, deoxynivalenol (Ia - also called vomitoxin), has been of considerable interest because it is of widespread occurrence and for example was present at 1-5 mg/kg in a high proportion of the 1980 Ontario and Quebec wheat harvest. Deoxynivalenol is considered to be of relatively low toxicity but other metabolites, some as yet uncharacterised, merit close examination.

Under conditions where heavily moulded cereals form an appreciable part of the diet, toxic effects may be severe. Consumption of *Fusarium graminearum* moulded maize has been correlated with a high incidence of human oesophageal cancer in the Transkei (Marasas *et al.* 1979) and China (Cheng *et al.* 1985). It is considered that the Alimentary Toxic Aleukia syndrome (which was the cause of death of many thousands of people in the Ukraine during the early and mid 1940s) was due to the consumption, enforced by famine, of heavily moulded grain containing trichothecene toxins (Ueno *et al.* 1972).

II. Toxin structures

More than 70 individual trichothecenes have been characterised to date. However, only deoxynivalenol and nivalenol have been found to any significant extent in naturally-contaminated commodities, although several others, including T-2 toxin and diacetoxyscripenol (DAS), occur on a more limited scale.

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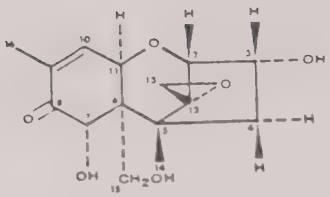

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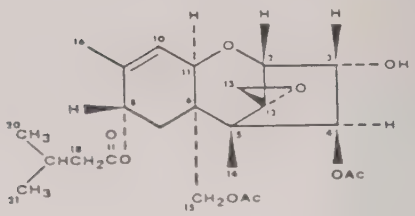

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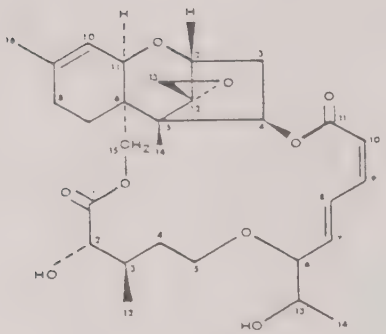
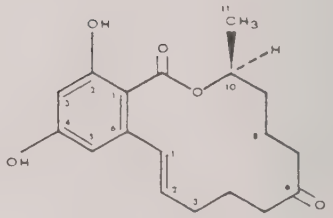
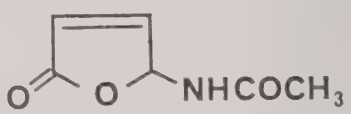


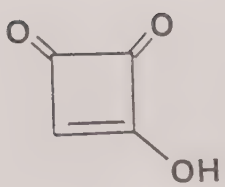
1c. Roridin A



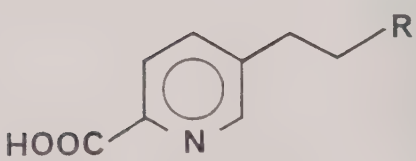
1d. Zearalenone



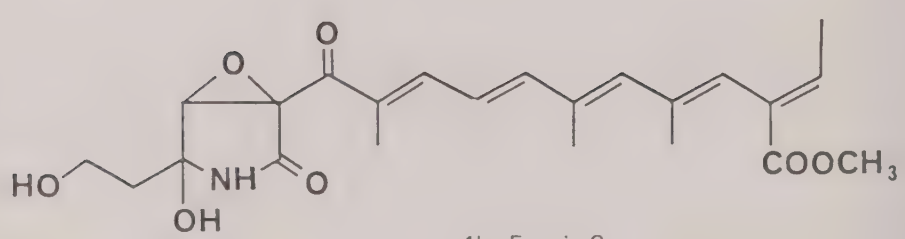
1f. Butenolide



1e. Moniliformin



1g. Fusaric acids



1h. Fusarin C

Fig. 1 Structures of some *Fusarium* toxins.

The large number of toxins arises because the trichothecene skeleton may be oxygenated at up to five different positions, and where hydroxyls are present these can be acylated with simple acids or with further functionalised long-chain diacids to produce macrocyclic esters. The simple esters form two groups, distinguished by the presence or absence of a carbonyl at C8. These groups are exemplified by deoxynivalenol (Ia) and T-2 toxin (Ib) respectively. The macrocyclic trichothecenes, which include roridin A (Ic), form a third type. Several books concerned with trichothecenes have appeared recently (Ueno 1983, Kurata & Ueno 1984, Lacey 1985).

Other *Fusarium* toxins show a wide variety of structures. These include zearalenone (Id), moniliformin (Ie), butenolide (If), fusaric acids (Ig) and fusarin C (Ih). Of these, extensive data on properties and occurrence are available only for zearalenone (Yoshizawa 1983). New metabolites are frequently discovered (Greenhalgh *et al.* 1986) and reports in the literature suggest that there are other as yet unidentified toxins present in crude fungal extracts (Gelderblom *et al.* 1984, Burmeister *et al.* 1980).

III. Factors affecting the formation and degradation of toxins

Little is known about toxins other than the trichothecenes. Canadian workers have put considerable effort into optimising the conditions for biosynthesis of deoxynivalenol. Toxin production *in vitro* on cereal substrates by field isolates of *F. graminearum* was shown to depend on the isolate, the substrate, moisture content, time of incubation, pH, oxygen and carbon dioxide concentrations and size of the culture flask (Greenhalgh *et al.* 1983). Many of these parameters also affect the formation of toxins in liquid culture (Miller *et al.* 1983).

It has been suggested that under natural conditions *Fusarium* species fall into two distinct chemotypes, producing either 3-oxygenated toxins (deoxynivalenol) or 3,4-oxygenated toxins (nivalenol) but not both. Thus Ichinoe *et al.* (1984) found that of 285 Japanese strains of *F. graminearum* and *Gibberella zeae* (the perfect stage of the fungus) examined, 43 produced deoxynivalenol and 131 nivalenol. None produced both toxins. However, Yoshizawa (1984) found two out of 43 Japanese isolates produced both these toxins, while 21 gave just deoxynivalenol, and 20 only nivalenol. There were clear regional differences in incidences of the two chemotypes in Japan (Ichinoe *et al.* 1984) and this finding is reflected elsewhere, for example in North America, where no nivalenol has been found (Scott 1984). Most *Fusarium* isolates will produce zearalenone in culture and some elaborate significant amounts of related oestrogenic compounds (Richardson *et al.* 1985).

Some cereal cultivars have resistance to infection by *Fusarium* species, and even when these plants do become infected they produce lower amounts of deoxynivalenol. In resistant cereals, the ratio of fungal biomass (ergosterol) to toxin was at least five times greater than in susceptible cultivars. It was suggested that resistant cultivars produce factors that prevent synthesis or promote degradation of the trichothecene (Miller *et al.* 1985). In both resistant and susceptible cultivars much of the toxin was present in the outer parts of the grain, with ratios of deoxynivalenol concentrations in the chaff and kernels of between 5 and 10. The relationship between deoxynivalenol concentration and grading factors for hard red winter wheat have been examined. Perhaps unsurprisingly, the most significant correlation found ($r = 0.75$) was with percentage of fungal damaged kernels (Shotwell *et al.* 1985).

The in-field preharvest formation of toxins has been examined in maize inoculated with *F. graminearum* (Miller *et al.* 1983). Deoxynivalenol production closely followed that of viable biomass, while zearalenone levels increased considerably only after a sharp decline in isolable colony-forming units. 15-Acetyldeoxynivalenol was also produced but in small amounts, in contrast to the results obtained on liquid culture of this mould. The results supported the suggestion that the acylated toxin is a precursor to deoxynivalenol. High deoxynivalenol concentrations were observed in soft winter wheat naturally infected in the field with *F. graminearum* two weeks before harvest, when the moisture content of the cereal was still around 50%. A subsequent 90% or greater reduction in toxin content at harvest time was observed in four separate fields. The metabolic fate of this lost toxin remains unknown (Scott *et al.* 1984).

Trichothecene production *in vitro* has been shown to be influenced by the presence of certain agricultural and food chemicals. The fungicide tridemorph exhibited a bimodal effect upon T-2 toxin formation (Moss & Frank 1985). Very low concentrations markedly repressed the toxin but at biocide levels of 30 mg/kg, production was enhanced by a factor of ten although biomass formation was reduced by one-third. Sub-inhibitory concentrations of the food preservative sorbic acid increased by up to 34-fold the production of T-2 toxin by *F. acuminatum* on maize meal (Gareis *et al.* 1984).

The stability of trichothecenes to heat is remarkably high; after 30 min at 150°C, only 10% of T-2 toxin and 20% of deoxynivalenol was degraded. Stability in boiling water was even greater (Kamimura *et al.* 1979). A gradual decline was observed in deoxynivalenol concentrations in a whole wheat flour sample stored at -20°C, with a 17% loss over 18 months (Gilbert, Sharman & Shepherd, unpublished results) but there are conflicting reports concerning trichothecene stability in compounded feed samples. Karppanen *et al.* (1985) found bioassay and chemical evidence for complete

loss of toxins including T-2 and deoxynivalenol from a range of feeds over 3-6 months at +4°C. El-Banna *et al.* (1983) established that deoxynivalenol was stable in poultry feed both at -20°C and at ambient temperatures (> 10°C) for at least 19 weeks. Differences in microbiological load and water content of samples leading to variations in enzyme activity could explain these results.

IV. Toxicology

Characteristic animal symptoms of trichothecene poisoning include feed refusal, emesis and haemorrhaging. Typically, there is considerable damage to proliferating cells of haematopoietic tissues in the bone marrow, spleen, thymus and lymph nodes (Rosenstein *et al.* 1979). White blood cells counts are reduced considerably. The toxicity of the trichothecenes is dependent upon the presence of the epoxide and compounds where this group has been removed show no biological activity (Bamburg 1976). The relative toxicity of individual compounds varies with the pattern of substitution of the trichothecene skeleton, in terms of both position and type of oxygenation and also of the extent of acylation. The Canadian authorites have determined a tentative tolerable deoxynivalenol intake for adults of 3 μg/kg body weight/day (Scott 1984).

Table 1. *A comparison of the toxicities of trichothecene mycotoxins to mice and in tissue culture*

Compound	LD_{50}[a] (Mice, mg/kg) i.p.	p.o.	Tissue culture (MIC[b], ng/ml)
verrucarin A	0.5		0.1
T-2 toxin	5.2	10.5	5
nivalenol	4.1		500
deoxynivalenol	70	46	2000

[a]Data from Ueno (1983).
[b]Shepherd, Mortimer & Gilbert, unpublished results.

The correlation between oral toxicity *in vivo* and minimum inhibitory concentrations (MIC) towards isolated cells (Table 1) is not good, although it should be noted that the LD_{50}data were obtained under varying conditions and are therefore not strictly comparable. It is found that 90-99% of an orally-administered dose is immediately eliminated in the urine and faeces (Yoshizawa *et al.* 1981). The major metabolite of DAS *in vitro* was a partially deacetylated glucuronide. Cytochrome oxidation, epoxide hydrolysis and glutathione conjugation products could not be found (Roush *et al.* 1985). Deacylation and glucuronidisation also appear to be important *in vivo* detoxification routes (Yoshizawa *et al.* 1981; Corley *et al.* 1985).

The sensitivities of a range of cultured cell lines to a given trichothecene are quite similar but animal responses vary widely, not only from species to species and strain but also depending upon the state of health and biochemistry of the individual animal. Poultry and cattle are in general resistant to trichothecenes, tolerating 50 and 10 mg/kg respectively of deoxynivalenol in the diet (probably because of detoxification in the gut) whereas early reports suggested that reduced consumption by pigs of naturally-contaminated feeds was found at a deoxynivalenol concentration of 0.3 mg/kg with emesis likely at a dose of 0.1-0.2 mg/kg body weight (Romer 1983).

However, it is probable that these effects on pigs are associated with components of contaminated feed other than deoxynivalenol. Naturally-contaminated feeds containing a given concentration of deoxynivalenol are both more toxic and more likely to be refused than diets prepared with similar amounts of the purified compound (Young *et al.* 1983), while a recent feeding trial in pigs at 10.5 mg/kg of toxin in the feed (from corn specially infected to produce large amounts of deoxynivalenol) showed a marked decrease in feed intake but no vomiting and no other toxic effects which could not be attributed to the reduced feed consumption (Lun *et al.* 1985). Rats on a diet incorporating 20 mg/kg of highly purified deoxynivalenol were less efficient in converting feed into body mass but few clinical signs of toxicity were observed (Morrissey *et al.* 1985).

The trichothecenes inhibit eucaryotic protein synthesis via at least two different mechanisms, binding reversibly to peptidyl transferases and in many cases causing dissociation of polysomes (Bamburg 1976). The minimum inhibitory concentrations are similar to those found to be cytostatic in tissue culture (Table 1). Immunofunction cells appear to be particularly sensitive, and lymphocyte protein synthesis, DNA synthesis and response to lectins, for example, are substantially depressed at concentrations of toxins which have no apparent effect upon other tissues. *In vivo,* trichothecenes cause marked weight loss with respect to controls in both the thymus and the spleen whereas liver or kidneys remain at their normal sizes (Sato *et al.* 1978). Roush *et al.* (1985) have indicated that the

glucuronide conjugate formed on metabolism of DAS is a poor inhibitor of protein synthesis compared to DAS itself.

There are reports of sex differences in responses to trichothecenes. Deoxynivalenol affected cell proliferation of crypt cells of the jejunum, red pulp cells of the spleen and basal cells of the oesophageal epithelium in male rats while females were consistently unaffected (Lok *et al.*, unpublished results). Enterohepatic recirculation of T-2 in mice has been observed (Matsumoto *et al.* 1978). Pigs receiving 0.15 mg/kg [^{3}H]T-2 intravascularly retained 1-13% of the dose in the gall bladder and glucuronides accounted for 77% of the total metabolite residues in the bile (Corley *et al.* 1985).

Moniliformin, butenolide, zearalenone and trichothecenes were all found not to be mutagenic in short-term bacterial assays, both with and without activation (Wehner *et al.* 1978). Other reports have indicated that deoxynivalenol was not mutagenic to Chinese hamster lung cells in an hepatocyte-mediated assay (Rogers & Heroux-Metcalf, 1983) and this toxin did not cause abnormal DNA synthesis in primary cultures of rat hepatocytes (Bradlaw *et al.*, 1985). However, Ueno *et al.* (1971) found that nivalenol and fusarenon-X were mutagenic in a yeast cell system. T-2 has been shown to induce single-strand breaks in the DNA of lymphoid organs, under conditions where no damage was observed with liver DNA, and rats developed leukaemia after long-term exposure to T-2 (Lafarge-Frayssinet *et al.* 1981).

Neither T-2 nor DAS caused increases in sister chromatid exchanges (SCE) in human lymphocytes, but zearalenone had a weak enhancing effect on SCE frequency at concentrations in the medium of greater than 2.5μg/mL (Cooray 1984). Embryotoxicity (without apparent maternal toxicity) was noted in mice intubated with purified deoxynivalenol at doses of 2.5 mg/kg or greater, with no adverse effects observed at 0.5 mg/kg (Khera *et al.* 1982). The immunological and other consequences of chronic exposure to low levels of trichothecenes in the diet are not adequately established and remain a cause for concern (Otokawa 1983).

Zearalenone has primarily an oestrogenic effect, causing reproductive abnormalities in farm animals, pigs in particular being susceptible to feed concentrations as low as 0.5 mg/kg. Sows receiving 5 mg/day throughout the last month of pregnancy produced litters with stillborn pigs or pigs with 'splayleg' incoordination of the hind legs (Bennett *et al.* 1978). Other *Fusarium* toxins are less well studied. Moniliformin and butenolide (Burmeister *et al.* 1980, and references cited therein) have, respectively, high and moderate acute toxicities. Butenolide inhibited the response to lectins of mouse spleen cells at concentrations of 20-100 ng/mL (Lafarge-Frayssinet *et al.* 1979). Fusarin C has been demonstrated to be mutagenic (Cheng *et al.* 1985, and references cited therein). Grains and other commodities frequently contain mixtures of toxins - Gelderblom *et al.* (1984) reported one sample of maize to be contaminated with moniliformin, zearalenone, deoxynivalenol and fusarin C - and possible synergism in the toxicity of such mixtures requires evaluation.

V. Analytical approaches

The analysis of *Fusarium* mycotoxins has been reviewed (Gilbert 1984, Mirocha *et al.* 1977). For trichothecenes, both efficient sample extraction and cleanup and also the development of specific and sensitive detection have proved particularly difficult. Trichothecenes are not easily extracted from naturally contaminated cereals (Trenholm *et al.* 1985) and the wide differences in polarities of individual members of the group means that single universal procedures (efficient for both T-2 toxin and nivalenol, for example) are not feasible. Extensive cleanup is required due to the lack of suitable specific chromatographic detection systems. It is particularly difficult to obtain good recoveries of nivalenol and adequate methods for this toxin have only just become available (Tanaka *et al.* 1985). Deoxynivalenol can be determined by a TLC method utilising a reaction with aluminium salts to produce a blue fluorescence (Trucksess *et al.* 1984), and, whilst other more general TLC visualisation reagents based on reaction with the epoxide group have been developed (Takitani *et al.* 1979), for biological samples these procedures are prone to interferences.

Capillary column GC is frequency employed as the determination stage after formation of either the trimethylsilyl (TMS) or heptafluorobutyryl (HFB) derivatives. However, despite lengthy cleanup there are still often numerous unassigned peaks present in the chromatogram, and for unequivocal confirmation of identity combined GC-MS is necessary. HPLC has not been the method of choice for trichothecenes as the low-wavelength UV detection required is both relatively insensitive and also insufficiently specific in view of the complexity of sample residues. The native fluorescence of zearalenone and related metabolites makes these compounds amenable to TLC and HPLC analysis and reversed phase HPLC with fluorescence detection is now routinely employed enabling sensitivities of 2-50μg/kg to be achieved readily. The application of HLPC to mycotoxin analysis has been reviewed recently (Shepherd 1986).

Many of the other *Fusarium* mycotoxins have, to date, been relatively neglected in terms of development of analytical methodology. Natural-occurrence data is therefore not available, with the consequence that assessment of their significance is difficult. Gelderblom *et al.* (1984) have described

a method for fusarin C in maize, and shown it to co-occur with moniliformin, deoxynivalenol and zearalenone in one sample of Transkeian maize.

An ion-pair procedure for moniliformin has been developed recently (Shepherd & Gilbert 1986) and methods for determining macrocyclic trichothecenes are being devised, based on novel techniques such as supercritical fluid chromatography (Smith *et al.* 1985). For the future, immunoassay approaches hold the most promise, offering advantages in specificity, sensitivity and particularly speed. The viability of ELISA methods for trichothecenes has already been demonstrated (Kemp *et al.* 1986), but before they can be applied to routine screening they will have to be ring-tested against conventional chemical methods in order to demonstrate that comparable results can be obtained.

VI. Natural occurrence

The worldwide natural occurrence of deoxynivalenol in wheat and maize, and occasionally in other cereals, has been established through surveys carried out over several years in Canada (Scott 1984), USA (Hagler *et al.* 1984, Cote *et al.* 1984, Shotwell *et al.* 1985), South Africa (Marasas *et al.* 1979), UK (Gilbert *et al.* 1983; Osborne & Willis 1984), Finland (Karppanen *et al.* 1985), West Germany (Blaas *et al.* 1984) and Japan (Yoshizawa & Hosokawa 1983, Yoshizawa 1984). Reported levels have ranged from a few μg/kg up to 41.6 mg/kg (Cote *et al.* 1984), and, for example, a 1982 US survey which found 10% of wheat samples from one area to contain greater than 4 mg/kg (Shotwell *et al.* 1985) appears to be by no means exceptional for North American grains. Zearalenone has frequently been found to co-occur with deoxynivalenol often at mg/kg levels and occasionally as high as 35 mg/kg (Scott 1978), whilst nivalenol has also been found in European and Asian grains but has not apparently been detectable in samples from North America (Scott 1984).

T-2 toxin and other trichothecenes have been reported to occur sporadically in cereals often intended for use as animal feedingstuffs. The natural occurrence of moniliformin (Thiel *et al.* 1982) and fusarin C (Gelderblom *et al.* 1984) at mg/kg levels has been established in a limited number of South African maize samples, but extensive surveys of these toxins in grains are still required. Reports of the natural occurrence of macrocyclic trichothecenes are limited but satratoxins have been isolated from the bedding straw of sheep suffering from stachybotryotoxicosis (Harrach *et al.* 1983). Butenolide has been found at up to 0.43 mg/kg in Japanese cereals (Yoshizawa 1984).

A range of trichothecenes was found in marketed potato tubers infected with fusarial dry rot. Concentration of up to 140 mg/kg were found in the rotted areas and levels of about 5% of this in apparently healthy tissues from the same tuber. The majority of these tubers were of one specific cultivar. (Lafont *et al.* 1983).

VII. Fate of toxins in the food chain

Most studies on the fate of trichothecenes have been concerned with deoxynivalenol in wheat. On milling contaminated wheat, the toxin has been shown to be distributed throughout the products (Scott *et al.* 1983, Seitz *et al.* 1985) with an increase in concentration in the bran and a slight decrease in the white flour. When contaminated flour was made into bread, about 90% of the deoxynivalenol survived the baking process (Scott *et al.* 1983) although some isomerisation was observed (Greenhalgh *et al.* 1984). Studies on both commercial wet and dry milling of deoxynivalenol-contaminated maize showed that the toxin fractionated predominantly into the germ, gluten and steep liquors, products which were primarily utilised for animal feed (Gilbert *et al.* 1983), although lesser amounts were still found in components intended for human consumption. Deoxynivalenol has been detected in a variety of retail cereal products including cornflakes, corn flour, brans and mixed cereal baby foods (Scott *et al.* 1982). Similar distributions of toxins in milled cereals have been reported for experimental wet milling of T-2 toxin-contaminated maize (Collins & Rosen 1981) and for zearalenone-contaminated maize (Bennett *et al.* 1978).

Deoxynivalenol fed to chickens was not detectable in eggs or in the drumstick, breast, liver or gizzard (El-Banna *et al.* 1983). Similarly, T-2 toxin was not apparently carried over into chicken tissue, although both this compound and a metabolite, HT-2 toxin, were found at low concentrations in the liver shortly after ingestion. Subsequent clearance was rapid (Hofmann 1980).

T-2 and HT-2 toxins (together with their analogues hydroxylated at the 3′ position on the isovaleryl moiety), neosolaniol and other unidentified metabolites, were detected in cow's milk at a maximum total concentration of 60 ng/mL trichothecene after daily oral intubation of 180 mg T-2 for three days, with on the fourth day a dose of 157 mg [^{3}H]T-2 (Yoshizawa *et al.* 1981). This regime corresponded to about 35 mg/kg T-2 in the feed. By 72 hours after the administration of the radiolabel, 0.2% of the activity had been recovered in the milk and more than 98% in the urine and faeces. For deoxynivalenol, only extremely low concentrations ($<$4ng/mL) of free and conjugated toxin were detectable in cow's milk following intake of a single oral dose of toxin (Prelusky *et al.* 1984). However, it is known that deoxynivalenol is metabolised by rumen microorganisms to a de-

epoxidised compound (King *et al.*, 1984) and as yet the extent of excretion of this and other metabolites into milk has not been established.

Zearalenone is metabolised by rumen fluid (Kiessling *et al.* 1984) to α- and β-zearalenols. The same compounds were found in sow's milk where the animals had been fed a diet containing 40 mg/kg zearalenone. Young pigs fed on this milk suffered oestrogenic effects (Palyusik *et al.* 1980). Zearalenone was not destroyed in fermented maize; indeed because of the loss of soluble components, the concentration of toxin in the residual solids was doubled (Bennett *et al.* 1981). For other *Fusarium* mycotoxins, occurrence data is limited and no information is available on their fate in the food chain. It might be expected that nivalenol would behave analogously to deoxynivalenol and other trichothecenes, but if the macrocyclic members of this group or compounds such as moniliformin are found to be important contaminants of foods or feeds then similar milling and animal product carry-over studies will be required.

VIII. Conclusion

Fusarium moulds produce a wide range of known toxic metabolites and there are indications that others remain to be discovered, particularly from *F. moniliforme*. Fortunately many of the known compounds remain laboratory curiosities at the present time but, apart from those for the trichothecenes and zearalenone, analytical methods and therefore occurrence data are lacking. This work is urgently required. The presence of deoxynivalenol in cereals is widespread, although subject to regional variation, and the toxin is apparently less of a problem to indigenous crops in Europe than in North America and Japan.

Recent work has indicated that deoxynivalenol itself probably presents a lesser hazard than at first thought, but the high levels sometimes encountered and the possibility of the co-occurrence of other toxins ensure that it will remain of concern to regulatory bodies. For example, the disparity in toxicities which has been sometimes been noted between naturally-contaminated and artificially prepared diets containing the same concentrations of deoxynivalenol has suggested that it may be a marker for other more toxic *Fusarium* metabolites. The recent high level of interest in *Fusarium* toxins has answered many questions, but predictably opened up others of considerable importance.

References

Bamburg, J.R. (1976) Chemical and biochemical studies of the trichothecene mycotoxins. In *Mycotoxins and other fungal related food problems.* (J.V. Rodricks, ed.), 144-162. Washington; American Chemical Society.

Bennett, G.A.; Vandegraft, E.E.; Shotwell, O.L.; Watson, S.A.; Bocan, B.J. (1978) Zearalenone : distribution in wet-milling fractions from contaminated corn. *Cereal Chemistry* **55**, 455-461.

Bennett, G.A.; Lagoda, A.A.; Shotwell, O.L.; Hesseltine, C.W. (1981) Utilisation of zearalenone-contaminated corn for ethanol production. *Journal of the American Oil Chemists' Society* **58**, 974-976.

Blaas, W.; Kellert, M.; Steinmeyer, S.; Tiebach, R.; Weber, R. (1984) Untersuchung von Cerealien auf Deoxynivalenol und Nivalenol im unteren µg/kg-Bereich. *Zeitschrift für Lebensmitteluntersuchung und -forschung* **179**, 104-108.

Bradlaw, J.A.; Swentzel, K.C.; Alterman, E.; Hauswirth, J.A. (1985) Evaluation of purified 4-deoxynivalenol (vomitoxin) for unscheduled DNA synthesis in the primary rat hepatocyte-DNA repair test. *Food and Chemical Toxicology* **23**, 1063-1067.

Burmeister, H.R.; Grove, M.D.; Kwolek, W.F. (1980) Moniliformin and butenolide: effect on mice of high-level, long-term oral intake. *Applied and Environmental Microbiology* **40**, 1142-1144.

Cheng, S.J.; Jiang, Y.Z.; Li, M.H.; Lo, H.Z. (1985) A mutagenic metabolite produced by *Fusarium moniliforme* isolated from Linxian county, China. *Carcinogenesis* **6**, 903-905.

Collins, G.J.; Rosen, J.D. (1981) Distribution of T-2 toxin in wet-milled corn products. *Journal of Food Science* **46**, 877-879.

Cooray, R. (1984) Effects of some mycotoxins on mitogen-induced blastogenesis and SCE frequency in human lymphocytes. *Food and Chemical Toxicology* **22**, 529-534.

Corley, R.A.; Swanson, S.P.; Buck, W.B. (1985) Glucuronide conjugates of T-2 toxin and metabolites in swine bile and urine. *Journal of Agricultural and Food Chemistry* **33**, 1085-1089.

Cote, L.M.; Reynolds, J.D.; Vesonder, R.F.; Buck, W.B.; Swanson, S.P.; Coffey, R.T.; Brown, D.C. (1984) Survey of vomitoxin-contaminated feed grains in midwestern United States, and associated health problems in swine. *Journal of the American Veterinary Medical Association* **184**, 189-192.

El-Banna, A.A.; Hamilton, R.M.G.; Scott, P.M.; Trenholm, H.L. (1983) Nontransmission of deoxynivalenol (vomitoxin) to eggs and meat in chickens fed deoxynivalenol-contaminated diets. *Journal of Agricultural and Food Chemistry* **31**, 1381-1384.

Gareis, M.; Bauer, J.; von Montgelas, A.; Gedek, B. (1984) Stimulation of aflatoxin B_1 and T-2 toxin production by sorbic acid. *Applied and Environmental Microbiology* **47**, 416-418.

Gelderblom, W.C.A.; Theil, P.G.; van der Merwe, K.J.; Marasas, W.F.O.; Spies, H.S.C. (1983) A mutagen produced by *Fusarium moniliforme*. *Toxicon* **21**, 467-473.

Gelderblom, W.C.A.; Theil, P.G.; Marasas, W.F.O.; van der Merwe, K.J. (1984) Natural occurrence of fusarin C, a mutagen produced by *Fusarium moniliforme*. *Journal of Agricutural and Food Chemistry* 1064-1067.

Gilbert, J. (1984) The detection and analysis of *Fusarium* mycotoxins. In *The applied mycology of Fusarium.* (M.O. Moss; J.E. Smith, eds), 175-193; Cambridge. Cambridge University Press.

Gilbert, J.; Shepherd, M.J.; Howell, M.V. (1983) Studies on the fate of trichothecene mycotoxins during food processing. *Proceedings. Second European Conference of Food Chemistry*, 253-262.

Gilbert, J.; Shepherd, M.J.; Startin, J.R. (1983) A survey of the occurrence of the trichothecene mycotoxin deoxynivalenol (vomitoxin) in UK-grown barley and in imported maize by combined gas chromatography - mass spectrometry. *Journal of the Science of Food and Agriculture* **34**, 86-92.

Greenhalgh, R.; Neish, G.A.; Miller J.D. (1983) Deoxynivalenol, acetyldeoxynivalenol and zearalenone formation by Canadian isolates of *Fusarium graminearum* on solid substrates. *Applied and Environmental Microbiology* **46**, 625-629.

Greenhalgh, R.; Gilbert, J.; King, R.R.; Blackwell, B.A.; Startin, J.R.; Shepherd, M.J. (1984) Synthesis, characterisation and occurrence in bread and cereal products of an isomer of 4-deoxynivalenol (vomitoxin). *Journal of Agricultural and Food Chemistry* **32**, 1416-1420.

Greenhalgh, R.; Meier, R.-M.; Blackwell, B.A.; Miller, J.D.; Taylor, A.; ApSimon, J.W. (1986) Minor metabolites of *Fusarium roseum* (ATCC 28114). Part 2. *Journal of Agricultural and Food Chemistry* **34**, 115-118.

Hagler, W.M.; Tyczkowska, K.; Hamilton, P.B. (1984) Simultaneous occurrence of deoxynivalenol, zearalenone and aflatoxin in 1982 scabby wheat from the midwestern United States. *Applied and Environmental Microbiology* **47**, 151-154.

Harrach, B.; Bata, A.; Bajmocy, E.; Benko, M. (1983) Isolation of satratoxins from the bedding straw of a sheep flock with fatal stachybotryotoxicosis. *Applied and Environmental Microbiology* **45**, 1419-1422.

Hofmann, G. (1980) Untersuchung zum Carry-over von T-2 Toxin bei Hühnern. *Fleischwirtschaft* **60**, 1908-1910.

Ichinoe, M.; Hagiwara, H.; Kurata, H. (1984) Distribution of trichothecene- producing fungi in barley and wheat fields in Japan. In *Toxigenic fungi - their toxins and health hazard.* (H. Kurata; Y. Ueno, eds), 190-198. Tokyo; Kodansha / Elsevier.

Kamimura, H.; Nishijima, M.; Saito, K.; Yasuda, K.; Ibe, A.; Nagayama, T.; Ushiyama, H.; Naoi, Y. (1979) The decomposition of trichothecene mycotoxins during food processing. *Journal of the Food Hygienic Society of Japan* **20**, 352-357.

Karppanen, E.; Rizzo, A.; Berg, S.; Lindfors, E.; Aho, R. (1985) *Fusarium* mycotoxins as a problem in Finnish feeds and cereals. *Journal of Agricultural Science, Finland* **57**, 195-206.

Kemp, A.J.; Mills, E.N.C.; Morgan, M.R.A. (1986) Enzyme-linked immunosorbent assay of 3-acetyldeoxynivalenol applied to rice. *Journal of the Science of Food and Agriculture* (in press).

Khera, K.S.; Angers, G.; Vesonder, R.F.; Kuiper-Goodman, T. (1982) Embryotoxicity of 4-deoxynivalenol (vomitoxin) in mice. *Bulletin of Environmental Contamination and Toxicology* **29**, 487-491.

Kiessling, K.-H.; Pettersson, H.; Sandholm, K.; Olsen, M. (1984) Metabolism of aflatoxin, ochratoxin A, zearalenone and three trichothecenes by intact rumen fluid, rumen protozoa and rumen bacteria. *Applied and Environmental Microbiology* **47**, 1070-1073.

King, R.R.; McQueen, R.E.; Levesque, D.; Greenhalgh, R. (1984) Transformation of deoxynivalenol (vomitoxin) by rumen microogranisms. *Journal of Agricultural and Food Chemistry* **32**, 1181-1183.

Kurata, H.; Ueno, Y., eds (1984) *Toxigenic fungi - their toxins and health hazard.* Tokyo; Kodansha / Elsevier.

Lacey, J., ed. (1985) *Trichothecenes and other mycotoxins.* Chichester; Wiley.

Lafarge-Frayssinet, C; Lespinats, G.; Lafont, P.; Loisillier, F.; Mousset, S.; Rosenstein, Y.; Frayssinet, C. (1979) Immunosuppressive effects of *Fusarium* extracts and trichothecenes : Blastogenic response of murine splenic and thymic cells to mitogens. *Proceedings. Society for Experimental Biology and Medicine* **160**, 302-311.

Lafarge-Frayssinet, C.; Decloitre, F.; Mousset, S.; Martin, M.; Frayssinet, C. (1981) Induction of DNA single-strand breaks by T-2 toxin, a trichothecene metabolite of *Fusarium*. Effect on lymphoid organs and liver. *Mutation Research* **88**, 115-123.

Lafont, P.; Girard, T.; Payen, J.; Sarfati, J.; Gaillardin, M. (1983) Contamination de pommes de terre de consommation par les fusariotrichothecenes. *Microbiologie, Alimentation, Nutrition* **1**, 147-152.

Lok, E.; Nera, E.; Iverson, F.; Arnold, D.; Clayson, D. (unpublished results) The effect of deoxynivalenol (vomitoxin) on cell proliferation in the crypt cells, splenic red pulp cells and esophageal cells of rats.

Lun, A.K.; Young, L.G.; Lumsden, J.H. (1985) The effects of vomitoxin and feed intake on the performance and blood characteristics of young pigs. *Journal of Animal Science* **61**, 1178-1185.

Marasas, W.F.O.; Van Rensburg, S.J.; Mirocha, C.J. (1979) Incidence of *Fusarium* species and the mycotoxins deoxynivalenol and zearalenone in corn produced in oesophageal cancer areas in Transkei. *Journal of Agricultural and Food Chemistry* **27**, 1108-1112.

Matsumoto, H.; Ito, T.; Ueno, Y. (1978) Toxicological approaches to the metabolites of *Fusaria*, Part 12. *Japanese Journal of Experimental Medicine* **48**, 393-399.

Miller, J.D.; Taylor, A.; Greenhalgh, R.; (1983) Production of deoxynivalenol and related compounds in liquid culture by *Fusarium graminearum*. *Canadian Journal of Microbiology* **29**, 1171-1178.

Miller, J.D.; Young, J.C.; Trenholm, H.L. (1983) *Fusarium* toxins in field corn. I. Time course of fungal growth and production of deoxynivalenol and other mycotoxins. *Canadian Journal of Botany* **61**, 3080-3087.

Miller, J.D.; Young, J.C.; Sampson, D.R. (1985) Deoxynivalenol and *Fusarium* head blight resistance in spring cereals. *Phytopathologische Zeitschrift* **113**, 359-367.

Mirocha, C.J.; Pathre, S.V.; Christensen, C.M. (1977) Chemistry of *Fusarium* and *Stachybotrys* mycotoxins. In *Mycotoxic fungi, mycotoxins, mycotoxicoses* Vol. 1. *Mycotoxic fungi and chemistry of mycotoxins* (T.D. Wyllie; L.G. Morehouse, eds), 365-420. New York; Marcel Dekker.

Morrissey, R.E.; Norred, W.P.; Vesonder, R.F. (1985) Subchronic toxicity of vomitoxin in Sprague-Dawley rats. *Food and Chemical Toxicology* **23**, 995-999.

Moss, M.O.; Frank, J.M. (1985) Influence of the fungicide Tridemorph on T-2 toxin production by *Fusarium sporotrichioides*. *Transactions of the British Mycological Society* **84**, 585-590.

Osborne, B.G.; Willis, K.H. (1984) Studies into the occurrence of some trichothecene mycotoxins in UK home-grown wheat and in imported wheat. *Journal of the Science of Food and Agriculture* **35**, 579-583.

Otokawa, M. (1983) Immunological disorders [due to trichothecenes]. In *Trichothecenes - chemical, biological and toxicological aspects*,(Y. Ueno, ed.) 163-170. Tokyo; Kodansha/Elsevier.

Palyusik, M.; Harrach, B.; Mirocha, C.J.; Pathre, S.V. (1980) Transmission of zearalenone and zearalenol into porcine milk. *Acta Veterinaria Academiae Scientiarum Hungaricae* **28**, 217-222.

Prelusky, D.B.; Trenholm, H.L.; Lawrence, G.A.; Scott, P.M. (1984) Nontransmission of deoxynivalenol (vomitoxin) to milk following oral administration to dairy cows. *Journal of Environmental Science and Health B* **19**, 593-609.

Richardson, K.E.; Hagler, W.M.; Mirocha, C.J. (1985) Production of zearalenone, alpha- and beta- zearalenol, and alpha- and beta- zearalanol by *Fusarium* spp. in rice culture. *Journal of Agricultural and Food Chemistry* **33**, 862-866.

Rogers, C.G.; Heroux-Metcalf, C. (1983) Cytotoxicity and absence of mutagenic activity of vomitoxin (4-deoxynivalenol) in a hepatocyte-mediated mutation assay with V79 Chinese hampster lung cells. *Cancer Letters* **20**, 29-35.

Romer, T.R. (1983) Questions about vomitoxin remain unanswered. *Feedstuffs*, April 11, 30-31.

Rosenstein, Y.; Lafarge-Frayssinet, C.; Lespinats, G.; Loisillier, F.; Lafont, P.; Frayssinet, C. (1979) Immunosuppresive activity of *Fusarium* toxins. Effects on antibody synthesis and skin grafts of crude extracts, T-2 toxin and diacetoxyscirpenol. *Immunology* **36**, 111-117.

Roush, W.R.; Marletta, M.A.; Russo-Rodriguez, S.; Recchia, J. (1985) Trichothecene metabolism studies: isolation and structure determination of 15-acetyl-3-alpha-(1-beta-D-gluco-pyranosiduronyl)scirpene-3,4-beta-15-triol. *Journal of the American Chemical Society* **107**, 3354-3355.

Sato, N.; Ito, T.; Kumada, H.; Ueno, Y.; Asano, K.; Saito, M.; Ohtsubo, K.; Ueno, I.; Hatanaka, Y. (1978) Toxicological approaches to the metabolites of *Fusaria*. Part 13. *Journal of Toxicological Science* **3**, 335-356.

Scott, P.M. (1978) Mycotoxins in feeds and ingredients and their origin. *Journal of Food Protection* **41**, 385-398.

Scott, P.M. (1984) The occurrence of vomitoxin (deoxynivalenol, DON) in Canadian grains. In *Toxigenic fungi - their toxins and health hazard* (H. Kurata; Y. Ueno, eds), 182-189. Tokyo; Kodansha/Elsevier.

Scott, P.M.; Lau, P.-Y.; Kanhere, S.R. (1981) Gas chromatography with electron capture and mass spectrometric detection of deoxynivalenol in wheat and other grains. *Journal of the Association of Official Analytical Chemists* **64**, 1364-1370.

Scott, P.M.; Kanhere, S.R.; Lau, P.-Y. (1982) Methodology for trichothecenes. *Proceedings, Fifth IUPAC Symposium on Mycotoxins and Phycotoxins*, 44-47.

Scott, P.M.; Kanhere, S.R.; Lau, P.-Y.; Dexter, J.E.; Greenhalgh, R. (1983) Effects of experimental flour milling and breadbaking on retention of deoxynivalenol (vomitoxin) in hard red spring wheat. *Cereal Chemistry* **60**, 421-424.

Scott, P.M.; Nelson, K.; Kanhere, S.S.; Karpinski, K.F.; Hayward, S.; Neish, G.A.; Teich, A.H. (1984) Decline in deoxynivalenol (vomitoxin) concentrations in 1983 Ontario winter wheat before harvest. *Applied and Environmental Microbiology* **48**, 884-886.

Seitz, L.M.; Yamazaki, W.T.; Clements, R.L.; Mohr, H.E.; Andrews, L. (1985) Distribution of deoxynivalenol in soft wheat streams. *Cereal Chemistry* **62**, 467-469.

Shepherd, M.J. (1986) High performance liquid chromatography and its application to the analysis of mycotoxins. In *Modern methods of analysis and structure elucidation of mycotoxins* (R.J. Cole, ed.) New York; Academic Press (in press).

Shepherd, M.J.; Gilbert, J. (1986) A method for the analysis in maize of the *Fusarium* mycotoxin moniliformin employing ion-pairing extraction and high performance liquid chromatography. *Journal of Chromatography* (in press).

Shotwell, O.L.; Bennett, G.A.; Stubblefield, R.D.; Shannon, G.M.; Kwolek, W.F.; Plattner, R.D. (1985) Deoxynivalenol in hard red winter wheat: relationship between toxin levels and factors that could be used in grading. *Journal of the Association of Official Analytical Chemists* **68**, 954-957.

Smith, R.D.; Udseth, H.R.; Wright, B.W. (1985) Rapid and high resolution capillary supercritical fluid chromatography (SCF) and SCF/MS of trichothecene mycotoxins. *Journal of Chromatographic Sciences* **23**, 192-199.

Takitani, S.; Asabe, Y.; Kato, T.; Suzuki, M.; Ueno, Y.(1979) Spectrodensimetric determination of trichothecene mycotoxins with 4-(*p*-nitrobenzyl)pyridine on silica gel thin layer chromatograms. *Journal of Chromatography* **172**, 335-342.

Tanaka, T.; Hasegawa, A.; Matsuki, Y.; Ishii, K.; Ueno, Y. (1985) Improved methodology for the simultaneous detection of the trichothecene mycotoxins deoxynivalenol and nivalenol in cereals. *Food Additive Contamination* **2**, 125-137.

Thiel, P.G.; Meyer, C.J.; Marasas, W.F.O. (1982) Natural occurrence of moniliformin together with deoxynivalenol and zearalenone in Transkeian corn. *Journal of Agricultural and Food Chemistry* **30**, 308-312.

Trenholm, H.L.; Warner, R.M.; Prelusky, D.B. (1985) Assessment of extraction procedures in the analysis of naturally contaminated grain products for deoxynivalenol (vomitoxin). *Journal of the Association of Official Analytical Chemists* **68**, 645-649.

Trucksess, M.W.; Nesheim, S.; Eppley, R.E. (1984) Thin layer chromatographic determination of deoxynivalenol in wheat and corn. *Journal of the Association of Official Analytical Chemists* **67**, 40-43.

Ueno, Y. (1983) General toxicology [of trichothecenes]. In *Trichothecenes - chemical, biological and toxicological aspects* (Y. Ueno, ed.), 135-146. Tokyo; Kodansha/Elsevier.

Ueno, Y.; Ueno, I.; Iitoi, Y.; Tsunoda, H.; Enomoto, M.; Ohtsubo, K. (1971) Toxicological approaches to the metabolites of *Fusaria*. Part 3. *Japanese Journal of Experimental Medicine* **41**, 521-539.

Ueno, Y.; Sato, N.; Ishii, K.; Sakai, K.; Enomoto, M. (1972) Toxicological approaches to the metabolites of *Fusaria*. Part 5. *Japanese Journal of Experimental Medicine* **42**, 461-472.

Wehner, F.C.; Marasas, W.F.O.; Thiel, P.G. (1978) Lack of mutagenicity to *Salmonella typhimurium* of some *Fusarium* mycotoxins. *Applied and Environmental Microbiology* **35**, 659-662.l

Yoshizawa, T. (1983) *Fusarium* metabolites other than trichothecenes. In *Trichothecenes - chemical, biological and toxicological aspects* (Y. Ueno, ed), 60-71. Tokyo; Kodansha/Elsevier.

Yoshizawa, T. (1984) Natural occurrence of *Fusarium* toxins in Japan. In *Toxigenic fungi - their toxins and health hazard* (H. Kurata; Y. Ueno, eds), 292-300. Tokyo; Kodansha/Elsevier.

Yoshizawa, T.; Mirocha, C.J.; Behrens, J.C.; Swanson, S.P. (1981) Metabolic fate of T-2 toxin in a lactating cow. *Food and Cosmetics Toxicology* **19**, 31-39.

Yoshizawa, T.; Hosokawa, H. (1983) Natural cooccurrence of deoxynivalenol and nivalenol, trichothecene mycotoxins, in commercial foods. *Journal of the Food Hygiene Society of Japan* **24**, 413-415.

Young, L.G.; McGirr, L.; Valli, V.E.; Lumsden, J.H.; Lun, A. (1983) Vomitoxin in corn fed to young pigs. *Journal of Animal Science* **57**, 655-665.

Spoilage and Mycotoxins of Cereals and other Stored Products.
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Natural Occurrence of Fungal Naphthoquinones in Cereals and Animal Feedstuffs

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Summary

The literature on fungal naphthoquinones is reviewed with emphasis on studies relevant to the possible occurrence of these metabolites in cereals and animal feedstuffs. Analytical methods developed for the determination of xanthomegnin, viomellein and vioxanthin in cereals and fungal cultures enabling detection of 10 μg/kg of these compounds in cereals are described.

Twenty selected samples known to contain citrinin and/or ochratoxin A were examined, and 12 were found to contain mixtures of the naphthoquinones with levels up to 1800 μg/kg of viomellein together with lower levels of xanthomegnin and vioxanthin. Examination of cultures of *Penicillium* strains isolated from naphthoquinone-negative cereal samples, showed that three out of 12 strains of species of the *P. cyclopium* group produced naphthoquinones in cultures on yeast extract sucrose (YES) medium.

Stationary culture of one of these strains produced xanthomegnin, viomellein and vioxanthin in approximately equal proportions on rice substrate and Czapek-1% corn steep liquor (CSL) medium. A mixed culture of 32 *Penicillium* isolates extracted from one sample of wheat found positive for naphthoquinones produced nearly 1 mg of each compound/100ml of Czapek-1% CSL medium in some culture flasks during 29 days at 25°C.

Further work is required to identify naphthoquinone producing strains of *Penicillium* and to establish the frequency with which these mycotoxins occur in stored cereals and animal feedstuffs in the United Kingdom.

I. Introduction

Ochratoxin A and citrinin are the mycotoxins found most frequently in samples of animal feedstuffs received at the regional laboratories of the UK Ministry of Agriculture, Fisheries and Food for mould and mycotoxin examination (Buckle 1983). In the UK there is little evidence to indicate any serious or widespread effect on livestock (Wells 1982). However, outbreaks of mycotoxic porcine nephropathy regularly occur in a number of European countries including Denmark (Krogh 1977), Sweden (Rutqvist *et al.* 1977), Netherlands (van der Molen *et al.* 1982) Hungary (Sandor *et al.* 1982) and Yugoslavia (Pepeljnjak & Cvetnić 1985). These outbreaks have been linked with the presence of

ochratoxin A in cereals and feedstuffs and in the kidneys, livers and blood especially of pigs. Ochratoxin A has also been authenticated as being at least a contributory factor in the occurrence of nephropathy in poultry (Hamilton *et al.* 1982).

In surveys carried out in Denmark, Sweden and Hungary, 25% to 39% of pigs' kidneys with nephrotic lesions contained detectable levels of ochratoxin A. Conversely, these findings imply that more than 60% of the kidneys were free of ochratoxin A. It was suggested by Hald *et al.* (1983) that other nephrotoxins might be implicated and these workers reported finding viomellein, a member of a group of fungal naphthoquinones, which also includes xanthomegnin, in one batch of barley associated with field cases of mycotoxic porcine nephropathy. Toxicological studies by Zimmerman *et al.* (1979 a,b) in which pigs were fed a diet inoculated with a culture of a known naphthoquinone-producing strain of *Aspergillus ochraceus*, had shown nephrotic effects similar to those produced by ochratoxin A.

The climate, storage conditions and fungal flora of stored cereals and feedstuffs in the UK are quite similar to those in Denmark and other parts of Europe. In view of this and of published data on the toxicology of naphthoquinones, a limited programme was initiated to investigate whether naphthoquinones could be detected in UK samples of cereals and feeds and, if so, to what extent they occur.

II. Literature review of the fungal napthoquinones

It has been known for many years that various species of *Trichophyton* and *Microsporum* fungi produce coloured pigments in culture. As early as 1929, Tate reported some properties of these pigments, but it was not until 1957 that both Mier (1957) and Wirth *et al.* (1957) coincidentally reported the isolation and separation of three crystalline coloured pigments from several strains of *T. rubrum*. Five years later, Blank *et al.* (1962) isolated and purified an orange compound from a culture of *T. megninii*. This compound was named xanthomegnin. There was strong evidence that this compound was the same as that previously obtained by Wirth *et al.* (1957). Just & Day (1963) examined chemical and physical properties of the new compound and proposed a chemical structure. A slightly modified form of this structure now generally accepted is shown in Fig. 1. Xanthomegnin was isolated from several strains of *T. rubrum* by Wirth *et al.* (1965).

Two further pigments were isolated by Blank *et al.* (1966) from *T. violaceum*. A brown-yellow crystalline substance was named vioxanthin and a dark red compound viopurpurin. Ng & Just (1968) investigated the structure and stereochemistry of these compounds. Interestingly, at this time Shotwell *et al.* (1969) reported the occurrence of ochratoxin A as a natural contaminant of corn for the first time, almost 14 years before a fungal naphthoquinone was to be detected in a cereal. A thin-layer chromatographic (TLC) method for studying the metabolites produced in the mycelium of *M. cookei* was published by Nozawa & Ito (1970). A number of coloured spots were observed on their TLC plates. Two of these were identified as xanthomegnin and luteosporin, a structurally related compound. Other spots which were present were probably viomellein and a number of analogues of xanthomegnin.

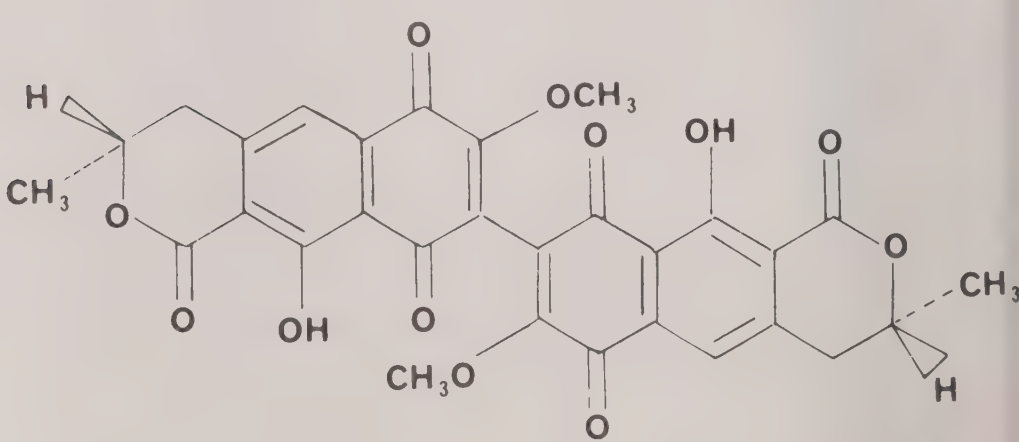


Fig. 1. Structure of xanthomegnin

A paper of particular significance for cereal storage science was that of Durley *et al.* (1975) which described the isolation of xanthomegnin and viopurpurin from cultures of *A. sulphureus* together with two new pigments, viomellein and rubrosulphin. The structure of viomellein is shown in Fig. 2. Xanthomegnin, viomellein and viopurpurin were also produced in cultures of *A. melleus*. As

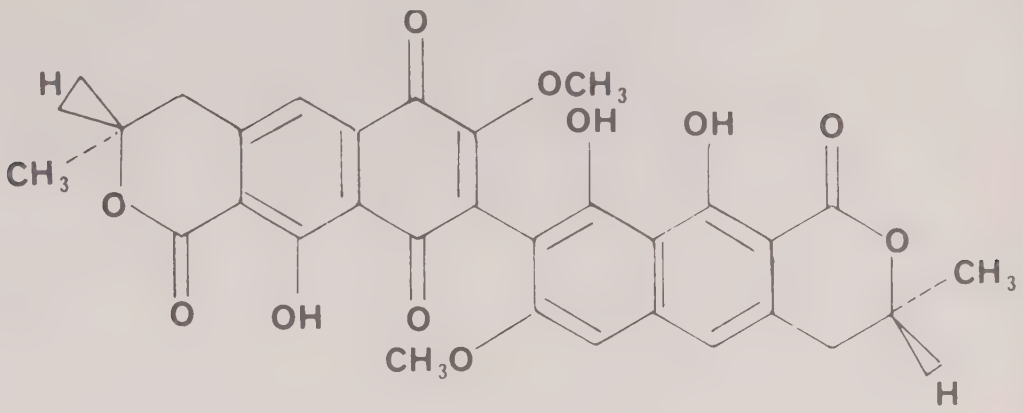


Fig. 2. Structure of viomellein

Penicillium and *Aspergillus* species commonly occur in stored cereals, the discovery that naphthoquinones could be obtained from aspergilli and shortly afterwards several species of *Penicillium* caused considerable interest and speculation as to whether they might be present in stored cereals or animal feedstuffs. Stack *et al.* (1977) found naphthoquinones produced by *P. viridicatum* and Stack & Mislivec (1978) described the detection of xanthomegnin and viomellein in cultures of *A. ochraceus, P. cyclopium* and *P. viridicatum.*

The search for suitable, sensitive analytical methods for determination of these compounds in foodstuffs was soon reflected in a series of publications describing analytical procedures. A High Performance Liquid Chromatographic (HPLC) method for determination of xanthomegnin in corn was published by Stack *et al.* (1978) with an estimated limit of detection of 750 μg/g and with recoveries for spiked standards of between 25% and 63%. However no cereal samples were reported positive for xanthomegnin. Ciegler *et al.* (1981) examined a number of strains of *P. viridicatum* and found in each case that strains which produced naphthoquinones gave neither ochratoxin A nor citrinin while those known to be ochratoxin or citrinin producers did not yield naphthoquinones. Results published by Frisvad & Filtenborg (1983) in their proposed classification of terverticillate penicillia based on profiles of mycotoxins and other secondary metabolites again suggested that strains of particular species produce either one or other class, but not both. However, in the same year Stack *et al.* (1983) examined a large number of isolates of strains of *A. ochraceus* obtained from green coffee beans and found that many of these produced not only xanthomegnin, viomellein and vioxanthin, but in many cases both ochratoxin A and ochratoxin B as well. A further HPLC method was published by Wall & Lillehoj (1983) with a claimed limit of detection of 300 μg/kg and with satisfactory separation of xanthomegnin and viomellein. However once again no naphthoquinones were obtained from natural substrates. The first authentic occurrence of viomellein in barley by Hald *et al.* (1983) has been discussed earlier.

Further analytical methods for the determination of xanthomegnin in corn have appeared (Carman *et al.* 1983, 1984). The use of electrochemical detection was described in the latter paper. This techniques is extremely sensitive for naphthoquinones and it was claimed reduced the potential limit of detection to well below that attained when using ultra-violet detection as employed in previous methods. Kuan *et al.* (1985) have formed a highly fluorescent derivative by reacting xanthomegnin with ammonia and hydrogen peroxide. As little as 0.1 ng can be detected using reverse phase HPLC and fluorescence detection. However problems were experienced due to background fluorescence when using spiked foods and feeds. Despite these improvements in methodology, until recently no further reports of the confirmed occurrence of naphthoquinones in cereal samples have appeared in the literature, although in a note describing chemical analysis for ochratoxin poisoning, Stahr *et al.* (1985) suggested that, in the examination of extracts of feeds and animal tissues, some of the extracts showed evidence of the presence of xanthomegnin. Preliminary studies on methods for detection of naphthoquinones in the UK were presented by Scudamore & Atkin (1984) and results describing discovery of a number of samples of wheat and barley and of one sample of oil seed rape containing xanthomegnin, viomellein and vioxanthin have been published recently (Scudamore *et al.* 1986).

No commercial source of naphthoquinones is currently available. However recent papers by Peterson & Grove (1983) and Jansen & Dose (1985) describe respectively the isolation and purification of xanthomegnin from *P. viridicatum* and of xanthomegnin, viomellein, rubrosulphin and viopurpurin from cultures of *P. viridicatum* and *A. ochraceus.*

The Association of Official Analytical Chemists (Scott 1985) has appointed an associate referee for xanthomegnin and related naphthoquinones and it is expected that interest in these fungal

metabolites will continue. The need for development and improvement of methods of analysis has been reiterated by Trenholm & Scott (1985).

A review of the toxicology of xanthomegnin and related quinone mycotoxins has been published by Mortimer & Ronaldson (1983). The main toxic effects produced by these compounds are considered to be due to photosensitization induced by fungal toxins. The results of feeding trials are presented, the major effects being on the kidney and livers of a number of species tested which have included mice, rats, guinea pigs and swine. Among biochemical effects mentioned, xanthomegnin is regarded as being a strong uncoupler of oxidative phosphorylation and can also bind to proteins and amino acids.

III. Development of analytical methods

1. Analytical considerations

Until recently, sensitive and selective methods of analysis have not been available for the determination of napthoquinones in food substrates. In developing analytical methods, many factors need to be considered, especially the efficiency of extraction of toxins from the food, clean-up of the extracts and detection and measurement of the compounds in purified extracts.

A particular problem with xanthomegnin and other naphthoquinones is their limited solubility in many solvents, especially in aqueous solutions. This can lead to difficulty in keeping the compounds in solution, especially if present in relatively large amounts or when, for instance, using aqueous-based reverse phase HPLC solvents. A second difficulty is that these naphthoquinones do not normally fluoresce when exposed to UV light. Fluorescence has provided the basis of extremely sensitive methods of detection for other toxins such as the aflatoxins, ochratoxins or citrinin. However for the naphthoquinones, the need to rely on UV absorption as the method for detection restricts the limits that can be obtained and increases the amount of interference seen during chromatography due to other co-eluting UV absorbing contaminants even after extensive clean-up. Thus until Carman *et al.* (1984) published a method using electrochemical detection, the best level of detection attainable was about 300 ng/g. This compares with limits of detection of less than 1 ng/g for ochratoxin A and citrinin, which can be readily achieved in clean extract solutions. This perhaps puts into perspective the reported occurrence of the naphthoquinones and ochratoxin and citrinin in field samples.

2. Extract and clean-up

An extraction procedure published by Carman *et al.* (1983) has been used as the basis of an improved method of analysis: 25 g of a well-mixed ground sample was added to a 250 ml Erlenmeyer flask with 100 ml of chloroform and 10 ml of 0.1M orthophosphoric acid. The flask was stoppered and shaken for 30 minutes before filtering the solution through anhydrous sodium sulphate. The solution was evaporated to near dryness (about 2 ml) under a stream of nitrogen before redissolving in chloroform and adding to a silica Sep-pak cartridge. The cartridge was rinsed with 8 ml of hexane: ethyl ether (3:1) which were discarded and the naphthoquinones finally eluted with chloroform : acetic acid (98:2). The solution was again evaporated to near dryness under nitrogen. Both ochratoxin A and citrinin also elute with the naphthoquinones under these conditions.

3. Chromotographic separation and detection

The solutions obtained after Sep-pak clean-up were sometimes sufficiently clean to use directly for determination either using TLC or reverse phase (RP) HPLC, but more frequently required additional semi-preparative (SP) clean-up. Fig. 3 shows alternative procedures which can be used to obtain extracts suitable for qualitative or quantitative determination.

TLC. Two-dimensional TLC was carried out on silica gel 60 aluminium plates, pre-treated with 10% tartaric acid in methanol and dried at 75°C for 15 minutes. A sample dissolved in chloroform : acetic acid (98:2) was spotted on the plate and developed in the first direction using benzene : methanol : glacial acetic acid (18 : 1 : 1) and in the second direction with toluene : ethyl acetate : 90% formic acid (6 : 3 : 1). Plates were allowed to dry in a fume hood and then examined under (a) daylight, (b) long-wave UV light and (c) long-wave UV light after treatment of the plate with ammonia vapour. The colours expected and the position of the spot obtained for xanthomegnin, viomellein, vioxanthin, ochratoxin A and citrinin are shown in Fig. 4.

When using TLC for SP clean-up, the maximum amount of sample was loaded on to the plate. After development, the spots were marked and the relevant areas cut out. The compounds were eluted from these areas with methanol : 0.2% orthophosphoric acid (70 : 30) for subsequent examination using RP-HPLC.

Normal phase (NP) HPLC. 100-200 µl aliquots of the sample solution dissolved in chloroform : acetic acid (98 : 2) after Sep-pak clean-up were injected into an HPLC fitted with a 25 cm HPLC column

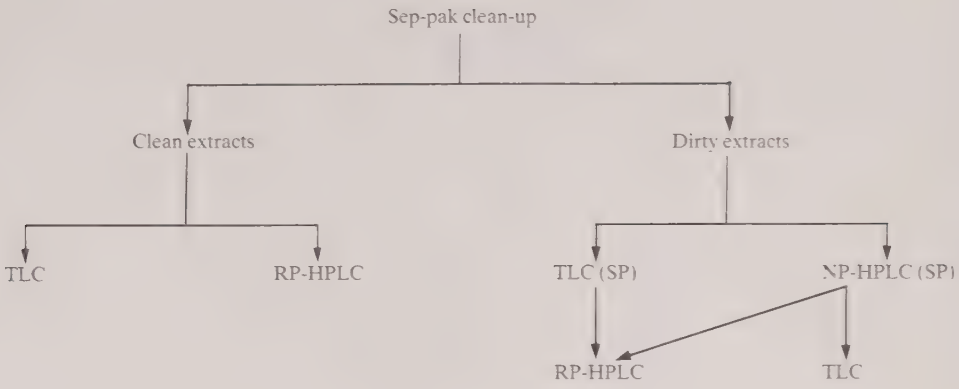
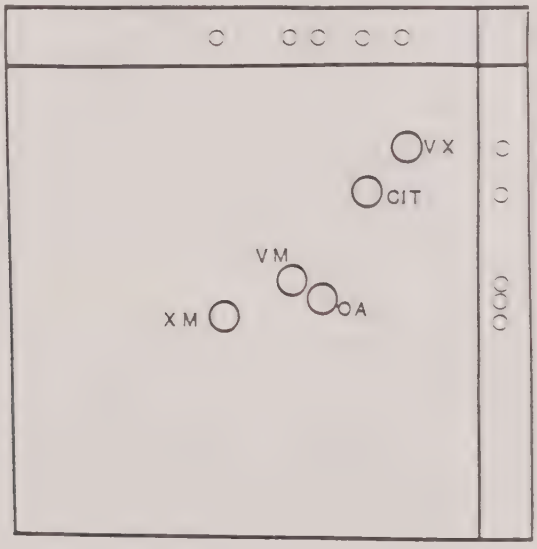


Fig. 3. Alternative procedures for the clean-up of cereal extracts containing naphthoquinone mycotoxins.

containing 5 μ or 10 μ silica. The solvent mixture used as the eluent was chloroform : acetic acid (98 : 2) and fractions eluting between 5 and 7 minutes (5 μ silica) monitored by UV detection at 390 nm were collected and bulked.
This solution was then divided only if necessary for subsequent dual determination by both TLC and RP-HPLC. The solution(s) was then evaporated to dryness under nitrogen and reconstituted in the appropriate solvent mixture.



	DAYLIGHT	LONG WAVE UV	$+NH_3$
VM = Viomellein	Yellow	Brown	Mauve-brown
XM = Xanthomegnin	Yellow	Brown	Mauve-brown
VX = Vioxanthin	–	Orange	Yellow-orange
OA = Ochratoxin A	–	Blue	Violet-blue
CIT = Citrinin	–	Yellow-green	–

Fig. 4. TLC of xanthomegnin, viomellein, vioxanthin, ochratoxin A and citrinin.

RP-HPLC. A suitably cleaned-up sample was dissolved in methanol : 0.2% orthophosphoric acid (70 : 30) and injected into a 25 cm 5 μ ODS RP-HPLC column using methanol : orthophosphoric acid at 1 ml/minute as eluent and UV detection at 390 nm. Fig. 5 shows the chromatogram obtained for a mixture of xanthomegnin and viomellein standards. Characteristic double peaks were obtained for both compounds. It has been suggested by Wall & Lillehoj (1983) for viomellein that these peaks are

diastereoisomers due to restricted rotation about the dimeric linkage. The greater resolving power of the column used here apparently indicates the existence of similar isomers of xanthomegnin and partial resolution of vioxanthin isomers has also recently been achieved.

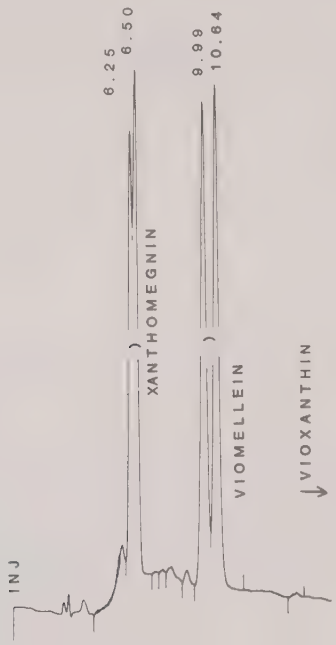


Fig. 5. HPLC of naphthoquinone standards on a reverse phase column.

Electrochemical detection. Limited experience with two commercial electrochemical detectors has suggested that significant improvement in sensitivity and selectivity should be possible using this method of detection. It seems likely that the lengthy clean-up procedures described above which are required to achieve limits of detection down to 10 ng/g may not be necessary once this technique has been fully evaluated.

IV. Examination of cereal samples for the presence of fungal naphthoquinones

Many of the samples received at Slough and found positive for mycotoxins are stored frozen for future reference, so that as methods are developed for other toxins, these samples can, if appropriate, be re-examined. From this stock, a few samples were selected on the basis that they had been found positive for at least ochratoxin and/or citrinin and gave considerable mould counts for *Penicillium* and *Aspergillus* spp. In general these samples had not as far as is known been the source of any problem in livestock. Interestingly, most of the samples subsequently found to be naphthoquinone-positive also contained sterigmatocystin, but this probably reflected the general mouldiness of those particular samples rather than any link with presence of the naphthoquinones.

Table 1 shows the levels of xanthomegnin, viomellein and vioxanthin and other mycotoxins found in some of the samples examined. The first sample examined was wheat M175. TLC of the crude extract showed an orange spot corresponding in position, and co-chromatographing with, vioxanthin. Direct injection of this extract into a RP-HPLC system suggested that both xanthomegnin and viomellein might also be present. After further clean-up and chromatography all the evidence from TLC and HPLC strongly suggested that all three compounds were indeed present. However, attempts to characterise these compounds by Fourier Transform Infra-Red analysis were inconclusive, probably due to the relatively low amounts present and the impurity of the extracts. The use of electrochemical detection however provided further evidence as peaks occurring with the same HPLC retention times as xanthomegnin and viomellein standards also had similar half-wave reduction potentials. It was considered that no further characterisation of the compounds could be made with this sample,especially as additional samples containing naphthoquinones had been identified. In all these the colours and R_F values obtained from TLC (Fig. 4), the characteristic split HPLC peaks and other properties provided convincing evidence that the naphthoquinones were present and at quite high levels in some of the samples.

To date, 12 out of a total of 20 samples selected for examination have proved positive for naphthoquinones. In each, all three compounds have been found present, except in barley M108.

Table 1. *Occurrence of naphthoquinones and other mycotoxins in cereals*

Commodity, Ref. No.	Mycotoxin, µg/kg					
	XM	VM	VX	OA	CIT	OTHER
Wheat, M175	200	1800	400	<50	270	ST, B_1
Wheat, M365	450	1800	800	3500	3000	ST
Wheat, M525	200	300	200	80	160	ST
Wheat, M110	600	800	250	–	+	–
Wheat, S274	–	–	–	210	30	–
Barley, S326	450	600	90	11000	1600	ST
Barley, M108	–	–	20	75	+	ST
Barley, S280	–	–	–	80	1	–
Oilseed rape, M504	+	+	40	–	4100	ST

XM = Xanthomegnin
VM = Viomellein
CIT = Citrinin
VX = Vioxanthin
OA = Ochratoxin A
B_1 = Aflatoxin B_1
ST = Sterigmatocystin

Only vioxanthin was detected in this sample, but this has been found to be the easiest compound to detect and it is possible that xanthomegnin and viomellein are also both present, but at levels below the current limit of detection. Of particular interest was the sample of oil seed rape which contained low levels of naththoquinones, a large amount of citrinin but no ochratoxin A.

Fig. 6 shows a chromatogram of the cleaned-up extract of barley S326 (Table 1). This barley contained exceptionally high levels of ochratoxin A, citrinin and other unidentified metabolites probably of fungal origin. However most of the interferences were effectively removed during the NP-HPLC clean-up stage, and UV detection at 390 nm provides relatively selective detection of xanthomegnin, viomellein and vioxanthin. Fig. 7 shows a chromatogram of a wheat extract. In this sample the amount of vioxanthin compared to xanthomegnin is considerably increased. In both chromatograms an unidentified peak occurred soon after the peaks for xanthomegnin. Its retention time did not correspond to two related naphthoquinones, rubrosulphin and viopurpurin for which standards were available.

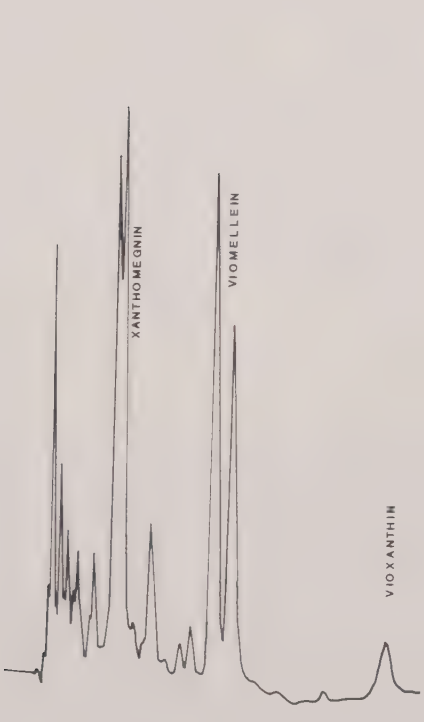


Fig. 6. *HPLC of an extract of barley, S326. Fig. 7. HPLC of an extract of wheat, S330.*

Presence of ochratoxin or citrinin does not give a reliable indication of the presence of naphthoquinones. For example, wheat M365 (Table 1) contains 10 times as much citrinin and nearly 100

times as much ochratoxin as wheat M175 although the levels of napthoquinones are similar. Both wheat M110 and oil seed rape M504 did not contain ochratoxin but were positive for naphthoquinones. It is likely that as improved analytical techniques become available more samples will be found to contain these naphthoquinones and possibly other related compounds.

V. Production of naphthoquinones in cultures of *Penicillium* species isolated from UK cereals

Studies to find strains of *Penicillium* species capable of producing naphthoquinones were carried out on 28 isolates of several *Penicillium* spp. extracted from 13 cereal samples available from other investigations. One advantage of working with these isolates was that they had been examined and identified at the Commonwealth Mycological Institute. Apart from information on which species isolated from UK cereal samples were capable of producing naphthquinones, identification of a good toxin-producing strain would make it possible to extract sufficient quantities of the myctoxins to use as chemical standards.

An aqueous spore suspension of each isolate was prepared and 1 ml aliquots added to flasks containing 100 ml of YES medium to give about 1 x 10^4 spores/ml. Six replicates were prepared for each isolate and were incubated in the dark at 25°C on rotary shakers, at about 140 rpm. Three of each set of flasks were left for 7 days and the other three for 14 days. After the required incubation period, the fungal pellets were filtered off and rinsed with sterile water. These pellets were transferred to a homogeniser beaker with 100 ml chloroform and 10 ml of 0.1M orthophosphoric acid. After homogenising for 15 minutes the resulting mixture was filtered through anhydrous sodium sulphate with washings, and the solution evaporated to near-dryness. The residue was made up in the appropriate solvent for either TLC or HPLC as described earlier. The liquid portion from the culture was partitioned directly against chloroform : phosphoric acid mixture and the lower chloroform layer was passed through anhydrous sodium sulphate and processed as above.

Out of the 28 cultures examined, of which 12 had been classified as *P. cyclopium* group species, three produced naphthoquinones. All were strains of *P. cyclopium* group species. In YES shake culture, vioxanthin was formed in largest amounts and production increased considerably between 7 and 14 days' incubation at 25°C (Table 2).

Table 2. *Production of naphthoquinones in the mycelium of cultures of Penicillium isolates.*

Isolate	Culture medium	Time, days	Total toxins/culture (μg) Xanthomegnin	Viomellein	Vioxanthin
S 274/1	Yes (Shake)	7	–	–	–
		14	2.8	–	2.2
S 280/1	Yes (Shake)	7	–	–	0.2
		14	–	–	1.7
S 331/1	Yes (Shake)	7	–	–	0.3
		14	0.2	0.4	3.5
S 331/1	Yes (Stationary)	10/20	–	–	–
S 331/1	Czapek-1% CSL	10	6	3	4
	(Stationary)	20	8	4	7
S 331/1	Rice (Stationary)	10	14	5	7
		20	11	4	6

For one of the strains, S331/1, alternative culture media and conditions were examined. Under static conditions no naphthoquinones were produced in YES whereas increased amounts of xanthomegnin, viomellein and vioxanthin were produced both in the Czapek-1% CSL and rice media. There did not appear to be any significant increase after 10 days. In all cases mycotoxins were only found in the mycelial pellets or mats, probably due to the insolubility of the mycotoxins in water.

Up to this time, isolates found to produce naphthoquinones in culture had been obtained mainly from samples of wheat or barley which did not originally contain naphthoquinones. It was decided to examine a recent sample in which naphthoquinones had been found. Wheat M365 (Table 1) contained high levels of xanthomegnin, viomellein and vioxanthin, in addition to ochratoxin A and citrinin and this sample was selected for study. A 10 g sample of wheat was homogenised in 90 ml of 0.2% agar diluent. The suspension was then serially diluted from 10^{-1} to 10^{-6}g/ml. At each dilution stage, 1 ml aliquots were taken and pipetted into sterile petri dishes to which cooled, liquid 2% malt agar containing penicillin and streptomycin were added. After the agar had set, the plates were incubated at 25°C for 7-10 days when they were inspected and the type of fungi identified and counted. A total of 32 colonies of *Penicillium* were present on the three replicate plates at 10^{-5}g/ml dilutions. These colonies were sub-cultured onto separate 2% malt agar phial slopes. One or two further sub-culturings were made until it was considered that the slopes represented pure cultures. A

flamed nichrome loop was then used to touch all 32 colonies on the three 10^5g dilution plates and this highly mixed inoculum was used to inoculate in several successive attempts the four different substrates: 50 g sterile moist rice and barley and 100 ml aliquots of Czapek-1%-CSL and YES. The pure culture slopes of the separate *Penicillium* isolates were retained while the analysis of substrates containing mixed inoculum were carried out, and also for subsequent determination of those strains which might be responsible for producing the mycotoxins detected from the mixed inoculum.

The cultures were incubated at 25°C under static conditions for 10 and 29 days before being examined for any naphthoquinone production. No ochratoxin or citrinin mycotoxins which might have been expected were found, but considerable amounts of viomellein, xanthomegnin and vioxanthin were produced on the rice, barley and in the mycelium in the Czapek-1% CSL medium after 10 days, growth (Table 3). However YES medium under static conditions again failed to produce significant amounts of toxins. Fig. 8 shows HPLC of the extract from one of the rice substrates inoculated with the 32 strains of *Penicillium* and Fig. 9 shows a similar chromatogram for the mycotoxins extracted from the Czapek-CSL medium, both after 10 days, growth at 25°C. Viomellein appears mainly as one peak and the reason for this is to be investigated but might suggest that only one isomer is produced in culture.

Table 3. *Production of naphthoquinones in mixed cultures of 32 Penicillium isolates from a naphthoquinone-positive sample of wheat, in stationary culture at 25°C.*

Culture medium	Time, days	Total toxins/culture (μg)		
		Xanthomegnin	Viomellein	Vioxanthin
Rice	10	250	250	100
	29	5	5	–
Barley	10	80	200	40
	24	10	10	–
YES	10	5	5	5
	29	–	–	3
Czapek-1% CSL	10	80	100	60
	29	80	400	500

After 29 days' growth, amounts of all three naphthoquinones had decreased considerably on rice and barley. However, in the Czapek medium, significant increases in the amounts of viomellein and vioxanthin had occurred. The medium favoured for toxin production was Czapek for this reason, and also because much cleaner extracts were obtained during subsequent processing and analysis. Considerable variation in amounts of toxins occurred between replicates and nearly 1 mg of toxin was produced in some culture flasks. Later work suggests that the efficiency in extracting toxins from mycelial pellets was probably low. Further work is now required to optimise conditions for production and extraction, to identify the active strains amongst the 32 isolates and to identify the species responsible.

VI. Conclusion

The presence of high levels of the fungal toxins xanthomegnin, viomellein and vioxanthin has been confirmed in a number of carefully selected mouldy cereal samples. Improvement in analytical methods and hence the lowering of limits of detection now makes it possible to carry out a systematic survey of the frequency of occurrence of these toxins relative to the occurrence of ochratoxin A and citrinin

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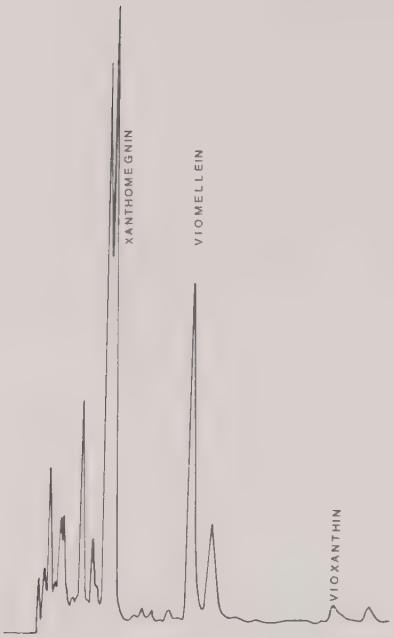

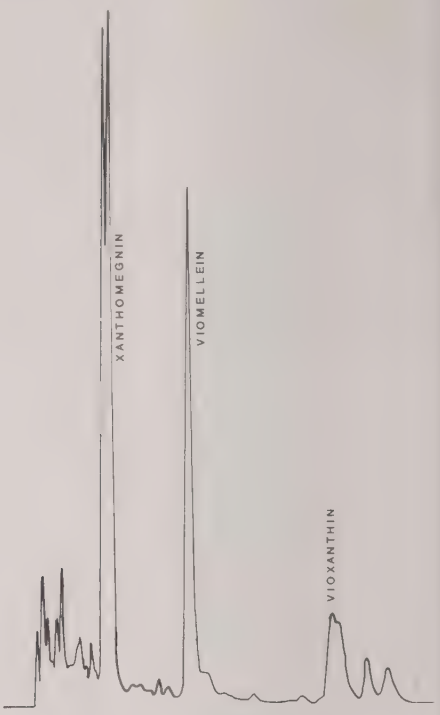

Fig. 8. HPLC of an extract from rice inoculated with a mixture of 32 strains of Penicillium species. Fig. 9. HPLC of an extract from mycelial growth in a culture of 32 strains of Penicillium species in Czapek-CSL medium.

References

Blank, F.; Day, W.C.; Just, G. (1962) Metabolites of pathogenic fungi. II. The isolation of xanthomegnin from *Trichophyton megninii* Blanchard 1896. *Journal of Investigative Dermatology* **40**, 133-137.

Blank, F.; Ng, A.S.; Just, G. (1966) Metabolites of pathogenic fungi. V. Isolation and tentative structures of vioxanthin and viopurpurin, two coloured metabolites of *Trichophyton violaceum*. *Canadian Journal of Chemistry* **44**, 2873-2879.

Buckle, A.E. (1983) The occurrence of mycotoxins in cereals and animal feed stuffs. *Veterinary Research Communications* **7**, 171-186.

Carman, A.S.; Kuan, S.S.; Francis, O.J.; Ware, G.M.; Gaul, J.A.; Thorpe, C.W. (1983) High pressure liquid chromatographic determination of xanthomegnin in grains and animal feeds. *Journal of the Association of Official Analytical Chemists* **66**, 587-591.

Carman, A.S.; Kuan, S.S,; Francis, O.J.; Ware, G.M.; Luedtke, A.E. (1984) Determination of xanthomegnin in grains and animal feeds by liquid chromatography with electrochemical detection. *Journal of the Association of Official Analytical Chemists* **67**, 1095-1098.

Ciegler, A.; Lee, L.S.; Dunn, J.J. (1981) Production of naphthoquinone mycotoxins and taxonomy of *Penicillium viridicatum*. *Applied and Environmental Microbiology* **42**, 446-449.

Durley, R.C.; MacMillan, J.; Simpson, T.J.; Glen, A.T.; Turner, W.B. (1975) Fungal products. XIII. Xanthomegnin, viomellein, rubrosulphin and viopurpurin pigments from *Aspergillus sulphureus* and *Aspergillus melleus*. *Journal of the Chemical Society, Perkin Transactions* **1**, 163-169.

Frisvad, J.C.; Filtenborg, O. (1983) Classification of terverticillate Penicillia based on profiles of mycotoxins and other secondary metabolites. *Applied and Environmental Microbiology* **46**, 1301-1310.

Hald, B.; Christensen, D.H.; Krogh, P. (1983) Natural occurrence of the mycotoxin viomellein in barley and the associated quinone-producing *Penicillia*. *Applied and Environmental Microbiology* **46**, 1311-1317.

Hamilton, P.B.; Huff, W.E.; Harris, J.R.; Wyatt, R.D. (1982) Natural occurrences of ochratoxicosis in poultry. *Poultry Science* **61**, 1832-1841.

Jansen, C.M.; Dose, K. (1985) Simultaneous isolation of xanthmegnin, viomellein, rubrosulphin, viopurpurin and brevianamide A by preparative HPLC. *Mycotoxin Research* **1**, 11-18.

Just, G.; Day, W.C. (1963) Metabolites of pathogenic fungi.III. The structure of xanthomegnin. *Canadian Journal of Chemistry* **41**, 74-79.

Krogh, P. (1977) Ochratoxin A residues in tissues of slaughtered pigs with nephropathy. *Nordisk Veterinaermedicin* **29**, 402-405.

Kuan, S.S.; Carman, A.S.; Francis, O.J.; Ware, G.M. (1985) Derivatisation of xanthomegnin for fluorometric determination. *Analytical Letters* **18** (**B7**), 837-846.

Mier, P.D. (1957) Pigments in *Trichophyton rubrum*. *Nature, London* **179**, 1084-1085.

Molen, E.J.; Egmond, H.P.; Paulsch, W.E. (1982) Chronic nephropathy in sows and the occurrence of ochratoxin A. *Proceedings of a Fourth Meeting on Mycotoxins in Animal Diseases*, 33-36. Reference Book 360. London; Ministry of Agriculture, Fisheries and Food.

Mortimer, P.H.; Ronaldson, J.W. (1983) Fungal-toxin-induced photosensitisation. In *Handbook of natural toxins*, Vol. 1. *Plant and fungal toxins*.(R.F. Keeler; A.T.Tu, eds), 361-419. New York; Marcel Dekker.

Ng, A.S.; Just, G. (1968) Metabolites of pathogenic fungi. VIII. On the structure and stereochemistry of xanthomegnin, vioxanthin and viopurpurin pigments from *Trichophyton violaceum*. *Canadian Journal of Chemistry* **47**, 1223-1227.

Nozawa, Y.; Ito, T. (1970) Separation of quinone pigments from *Microsporum cookei* by thin-layer chromatography. *Experimentia* **26**, 803-804.

Pepeljnjak, S.; Cvetnić, Z. (1985) The mycotoxicological chain and contamination of food by ochratoxin A in the nephropathic and non-nephropathic areas in Yugoslavia. *Mycopathologia* **90**, 147-153.

Peterson, R.E.; Grove, M.D. (1983) Isolation of xanthomegnin from *Penicillium viridicatum* by preparative high pressure liquid chromatography. *Applied and Environmental Microbiology* **45**, 1937-1938.

Rutqvist, L.; Björklund, N.-E.; Hult, K.; Gatenbeck, S. (1977) Spontaneous occurrence of ochratoxin residues in kidneys of fattening pigs. *Zentralblatt für Veterinaermedizin, Reihe A* **24**, 402-408.

Sandor, G.; Glavits, R.; Vajda, L.; Vanyi, A.; Krogh, P. (1982) Epidemiologic study of ochratoxin A - associated porcine nephropathy in Hungary. In *Proceedings, Fifth International IUPAC Symposium on Mycotoxins and Phycotoxins, Vienna*, 349-352.

Scott, P.M. (1985) Report on mycotoxins. *Journal of the Association of Official Analytical Chemists* **68**, 242-248.

Scudamore, K.A.; Atkin, P.M. (1984) Determination of ochratoxin A, citrinin and other co-occurring mycotoxins in animal feeding stuffs. *Proceedings of a Fifth Meeting on Mycotoxins in Animal and Human Health, Edinburgh 1984* (M.O. Moss; M. Frank, eds), 30-36. Guildford; University of Surrey.

Scudamore, K.A.; Atkin, P.M.; Buckle, A.E. (1986) Natural occurrence of the naphthoquinone mycotoxins, xanthomegnin, viomellein and vioxanthin in cereals and animal feed stuffs. *Journal of Stored Products Research* **22**, 81-84.

Shotwell, O.L.; Hesseltine, C.W.; Goulden, M.L. (1969) Ochratoxin A : occurrence as natural contaminant of a corn sample. *Applied Microbiology* **17**, 765-766.

Stack, M.E.; Eppley, R.M.; Dreifuss, P.A.; Pohland, A.E. (1977) Isolation and identification of xanthomegnin, viomellein, rubrosulphin and viopurpurin as metabolites of *Penicillium viridicatum*. *Applied and Environmental Microbiology* **33**, 351-355.

Stack, M.E.; Brown, N.L.; Eppley, R.M. (1978) High pressure liquid chromatographic determination of xanthomegnin in corn. *Journal of the Association of Analytical Chemists* **61**, 590-592.

Stack, M.E.; Mislivec, P.B. (1978) Production of xanthomegnin and viomellein by isolates of *Aspergillus ochraceus*. *Penicillium cyclopium* and *Penicillium viridicatum*. *Applied and Environmental Microbiology* **36**, 552-554.

Stack, M.E.; Mislivec, P.B.; Denizel, T.; Gibson, R.; Pohland, A.E. (1983) Ochratoxins A and B, xanthomegnin, viomellein and vioxanthin production by isolates of *Aspergillus ochraceus* from green coffee beans. *Journal of Food Protection* **46**, 965-968.

Stahr, H.M.; Demoto, M; Zhu, B.L.; Pfeiffer, R. (1985) Chemical analysis for ochratoxin poisoning. *Mycotoxin Research* **1**, 31-35.

Tate, P. (1929) The dermatophytes or ringworm fungi. *Biological Reviews* **4**, 41-75.

Trenholm, H.L.; Scott, P.M. (1985) Gaps in knowledge and recommendations for research identified by the Panel of Authors. In *Mycotoxins : a Canadian perspective*, 125-131. Research Council of Canada Publication No. 22848. Ottawa; NRCC.

Wall, J.H.; Lillehoj, E.B. (1983) High performance liquid chromatographic separation of xanthomegnin and viomellein. *Journal of Chromatography* **268**, 463-468.

Wells, G.A. (1982) Mycotoxic porcine nephropathy - a disease unreported in Great Britain. *Proceedings of a Fourth Meeting on Mycotoxins in Animal Diseases,* 29. Reference Book 360. London; Ministry of Agriculture, Fisheries and Food.

Wirth, J.G.; O'Brien, P.J.; Schmitt, F.L.; Sohler, A. (1957) The isolation in crystalline form of some of the pigments of *Trichophyton rubrum*. *Journal of Investigative Dermatology* **29**, 47-53.

Wirth, J.C.; Beesley, T.E.; Amand, S.R. (1965) The isolation of xanthomegnin from several strains of the dermatophyte, *Trichophyton rubrum*. *Journal of Investigative Dermatology* **9**, 47-53.

Zimmerman, J.L.; Carlton, W.W.; Tuite, J. (1979a) Mycotoxicosis produced in swine by cultural products of an isolate of *Aspergillus ochraceus*. I. Clinical observations and pathology. *Veterinary Pathology* **16**, 583-593.

Zimmerman, J.L.; Carlton, W.W.; Tuite, J. (1979b) Mycotoxicosis produced in swine by cultural products of an isolate of *Aspergillus ochraceus*. II. Clinicopathologic changes. *Veterinary Pathology* **16**, 702-709.

Spoilage and Mycotoxins of Cereals and other Stored Products.
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Problems Involved in the Determination of Aflatoxin Levels in Bulk Commodities

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I. Summary

The distribution of aflatoxins in groundnuts has been extensively studied and plans have been developed in the USA and the UK for monitoring the levels of aflatoxin in this commodity. Attempts have been made to assess the sampling plans using various statistical models. In determining the aflatoxin levels in bulk commodities errors can arise at several stages, for example, in sampling, sample preparation, and analysis. These errors and the attempts which are being made to limit their effects are discussed. Earlier workers have applied the negative binomial and compound poisson-gamma distribution to the distribution of aflatoxins in edible groundnuts, but with limited success. Data for aflatoxin levels in different batches of groundnuts and groundnut cake were found to give a better fit over a wider range using the Weibull distribution than the other distributions.

II. Introduction

With the report of aflatoxicosis in turkey poults and ducklings in 1960 and the subsequent discovery of the acute and chronic toxicities of aflatoxins, many countries decided to introduce regulations to control the levels of these toxins in human and animal feedingstuffs. Almost all agricultural commodities will support the growth of the aflatoxin-producing fungi *Aspergillus flavus* and *A. parasiticus*. Aflatoxins can be formed during the pre- and post-harvest stages of food production as long as a suitable environment for mould growth is provided. Different crops vary in their ability to support fungal colonisation because of differences in the chemical composition of each commodity.

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The incidence and magnitude of aflatoxin contamination varies with geographical and seasonal factors and also with the conditions under which the crop is grown, harvested, stored and transported. Climatic changes associated with temperature and humidity, in particular conditions of drought, can greatly influence the production of aflatoxins. With groundnuts, contamination occurs primarily in the field after harvest when the nuts are being dried. Both mechanical and insect damage, and excessive rain during the drying period all contribute to invasion by *A. flavus*. The level of contamination is largely determined by the extent to which mould growth is prevented at all stages of production and utilisation. To this end, effective methods have been devised to detect aflatoxins in low levels, to minimise the damage to food crops and to detoxify contaminated material (Coker *et al.* 1985).

The permitted concentration of aflatoxin varies quite widely depending on the country and the foodstuff and also whether the foodstuff is for human or animal use. As an example, maximum permitted levels for foods for human use in certain selected countries are listed in Table 1.

The EC issued a directive in 1976 restricting the amount of aflatoxin permitted in an animal feedstuff to levels not exceeding 50 μg/kg (Jewers 1982). More recently the European Parliament has endorsed a Commission proposal that maximum permitted levels of aflatoxin in raw materials used in animal feeds should be fixed and, where exceeded, should not be either imported or put to further use. The Parliament proposed 200 μg/kg at 12% moisture (not 300 μg/kg as suggested by the Commission), with a reduction to 100 μg/kg as soon as technically feasible (Hanson 1985). The UK Government is unlikely to increase the level it has set for imported groundnut cake (50 μg/kg) to the level proposed as this could lead to unacceptable levels of aflatoxin M_1 in milk.

Despite the maximum limits placed on aflatoxin contents by various countries, there are many problems associated with the determination of the level in a large consignment of a commodity. The determination of the aflatoxin content of a small sample can be achieved with a high degree of accuracy, but substantial errors can arise in obtaining a representative sample from a large batch of produce. It is quite possible for the analytical data to indicate that the commodity contains less than the permitted level of aflatoxin, whereas the mean value of the consignment may be in excess of the limit or, conversely, the data may indicate that the batch contains more than the permitted level, whereas the mean value may be less. The first case is associated with potential health hazards (consumer risk) and the latter with economic losses to the producer (producer risk). Discounts are often offered for the purchase of commodities which exceed the permitted level of aflatoxin and these discounts can have major financial implications for producing countries.

Because of the problems involved, sampling plans have been developed and evaluated in attempts to minimise the errors which can arise in producing a representative sample from a bulk lot (Whitaker *et al.* 1974). Some of the problems associated with sampling, sample preparation and analysis are discussed below.

Table 1. *Maximum permitted levels for aflatoxins in foodstuffs for human use*

Country	Maximum permitted level (μg/kg)	Product(s)
Canada	15 (total aflatoxins)	Edible nuts and nut products
Denmark	10 (total aflaxotoxins)	Groundnuts and groundnut products
India	60 (B_1)	Groundnut flour
Italy	50 (B_1)	Groundnuts and groundnut products
Netherlands	0 (detection limit of method, 5 μg/kg	Groundnuts and groundnut products
Sweden	5 (total aflatoxins) 0.05 (M_1)	Roasted peanuts Milk, specified milk products
U.K.	30 (total aflatoxins)	Imported nuts and nut products
U.S.A.	25 (total aflatoxins) 20 (total aflatoxins)	Raw unprocessed groundnuts Edible nuts and nut products
West Germany	10 (total aflatoxins) maximum 5 (B_1)	Edible nuts and nut products

III. Problems associated with sampling

Although limits are placed on the amounts of aflatoxins allowed in feedingstuffs, the determination of reliable analytical data has many difficulties. One major problem is obtaining a satisfactory sample

of the consignment under test. For example, with groundnuts relatively few kernels in any one lot are likely to be contaminated. Moreover, the level of aflatoxin will vary from one kernel to another from 0 to perhaps 300,000 μg/kg and the contaminated kernels will be randomly distributed throughout the lot. Variations in aflatoxin levels in individual groundnuts and groundnut sections have been recorded by Cucullu *et al.* (1960). They found that in groundnut samples of low contamination only about 0.24% of the individual kernels contained aflatoxin, while at higher concentration levels about 5% of the kernels were contaminated. Recently we have studied the variation in aflatoxin content found in different samples taken from a single batch of groundnuts; values ranging from 0 to 202 μg/kg were recorded (Table 2).

Table 2. *The aflatoxin contents of 20 subsamples taken from a 54 kg sample of groundnut kernels*

Aflatoxin content (μg/kg)					
202	5	0	39	0	
1	0	159	1	1	Av. content
16	0	46	14	0	31 μg/kg
0	94	0	43	0	

Velasco & Whitaker (1975) found similar large variations in the aflatoxin content of different sublots of commercial samples of contaminated cottonseed, and Cornelius & Maduagwu (1986) also found a highly skewed distribution of aflatoxin in palm kernels from West Africa.

Several sampling plans have been proposed for the examination of consignments of various commodities, for example those devised for groundnuts by the United States Department of Agriculture (USDA) (Dickens 1977) and the Tropical Development and Research Institute (TDRI) in the UK (Jewers 1982). In the USDA sampling procedure for groundnuts a 65 kg sample is collected from the batch to be tested, irrespective of size, by sampling every fourth sack, and this is sub-divided into three equal samples. From each of these, 1100 g sub-samples are prepared (A,B,C), which are divided into two (A1, A2, B1, B2, C1, C2). Both A samples are assayed for aflatoxin content and the mean of the two values calculated. If this is less than 16 μg/kg the sample is accepted, but if greater than 75 μg/kg the consignment is rejected. When the value lies between 16 and 75 μg/kg the two B samples are then assayed. If the mean value of A + B is less than 22 μg/kg the batch is accepted, but if greater than 38 μg/kg it is rejected. The two C samples are assayed when the mean value of the A + B samples is between 22 and 38 μg/kg. If the A + B + C samples have a mean aflatoxin level of less than 25 μg/kg the batch is accepted, but if greater than this it is rejected.

The TDRI sampling plan involves the use of a considerably smaller sample of 10.5 kg. This is made up by combining subsamples from at least 100 sacks from throughout a batch of up to 20 tonnes and, for batches greater than this, the number of subsamples is increased on a pro-rata basis. The 10.5 kg sample is then subdivided into three equal portions, each of which is reduced to a 350 g subsample. If one of these contains less than 10 μg/kg then the batch is accepted as containing 30 μg/kg, or less, of aflatoxin.

The sampling plans of both the USDA and the TDRI have been assessed with regard to their relative consumer and producer risks using the 1974 USA groundnut data (Jewers 1982). It was shown that the TDRI plan involved a higher consumer but a lower producer risk than the USDA plan. However, the TDRI method requires considerably smaller samples than that of the USDA and is, therefore, cheaper to implement.

The distributions of aflatoxin in three different batches of groundnuts were investigated both before and after roasting. From each batch, which varied from 15-20 tonnes, numerous subsamples were taken, each of which was analysed for its content of aflatoxin. The results obtained are given in Table 3.

Table 3. *Distribution of aflatoxin in batches of groundnuts before (B) and after roasting (R)*

Batch	Batch size (tonnes)	No. of sub-samples	Contaminated sub-samples (%)	Mean aflatoxin conc. (μg/kg)	Maximum aflatoxin conc. (μg/kg)
A (B)	20	200 × 100 g	2	1.0	100
A (R)	20	150 × 200 g	2	1.0	100
B (B)	20	200 × 100 g	6	40.0	4000
B (R)	20	200 × 100 g	3.5	10.0	2000
C (B)	15	200 × 100 g	1	10.0	1000
C (R)	15	200 × 100 g	1.5	1.0	100

These results show that although roasting appeared to reduce the aflatoxin content of batches B and C, the level in some subsamples was still significant. We have investigated a further 7 batches of processed groundnut products (roasted groundnuts and peanut butter) with sample sizes varying from 20 to 34 kg. The mean aflatoxin content of these samples varied from 0 to 10 μg/kg, but the percentage of contaminated subsamples did not exceed 3% and the maximum subsample concentration was 600 μg/kg in an individual pack.

Because of the skewed distribution of aflatoxin in any batch of commodity, it is essential that statistically-valid sampling procedures are used in order to estimate, with any degree of accuracy, the mean aflatoxin content of the batch. It is necessary to take many samples from any one consignment. Because of this, quality control can only be carried out effectively at the factory, both before and after processing, but before the product is released in small batches to the retail trade. At the retail level, surveillance is very difficult. An environmental health officer may analyse, for example, a jar of peanut butter, but, if the aflatoxin content is higher than the permitted level, a problem arises, because a single result has little significance. However, to obtain useful information, many jars from the same batch would need to be analysed and this is likely to be impossible.

Because of the need for proper sampling procedures, results obtained from small samples have to be regarded with suspicion. Kershaw (1985) accepted the reliable protocol based on statistical evaluation of the TDRI procedure, but considered that, from the small to medium sized importer's point of view, the cost required to generate large samples from each consignment is prohibitive. As a result, he accepted samples of typically 1-2 kg edible nuts on the assumption that they were representative of the parcels sampled. How this was achieved is not stated. Moreover, considering the variations in aflatoxin content found in different subsamples taken from any one batch (Tables 2 and 3), the value of Kershaw's data has to be doubted.

Problems are encountered in taking representative samples from a large consignment stored, for example, in the hold of a boat, which may contain 20,000 tonnes of the commodity; in a silo, which may store 2,000 tonnes; or in sacks in a store house. Attempts have to be made to obtain samples for analysis which can be regarded as representative of the whole lot. To achieve this, large samples are required which are then comminuted and subsampled. Ideally, for analytical purposes, the sample should be prepared either from material taken continuously during loading or unloading, or by mixing probe samples extracted from widely dispersed sacks. The larger the size of the particles in the commodity under test the greater the sample size required. For very small particles (e.g. milk, milk products) the minimum sample size is 500 g, for particles of intermediate size (e.g. finely ground meals and flours) it is 3 kg, for small grains (e.g. wheat, barley) 5 kg, for intermediate grains (e.g. maize, cottonseed) 10 kg, and for large grains (e.g. groundnuts, tree-nuts) 20 kg. Speed is necessary in taking the samples and analysing for aflatoxin content so that commercial decisions can be taken before release of the commodity.

IV. Problems associated with sample preparation

In sample preparation it is assumed that the final subsample taken for analysis is representative of the original sample collected from the batch. In reality the various stages of sample preparation, including dividing, comminution and mixing, can lead to significant variations in mycotoxin levels in the different subsamples. As an example of the problems involved, after comminution the distribution of granular material throughout the mixture will nearly always deviate from the mean value. Different milling methods can also lead to variations in the distribution of particles throughout the sample. Apelt *et al.* (1985) studied different methods of sample division and found that the use of mechanical dividing instruments can increase accuracy and prevent errors due to inexpert handling of the samples. Careful handling of the sample material, especially repeated thorough mixing after subsampling to reverse any separation that may have occurred, ensured that variations were kept at equally low levels for materials with a tendency to demix as for mixtures without this problem. If sufficient care is not taken, significant variations occur between subsamples. Because of the highly skewed distribution of aflatoxin in a single batch of a commodity, the problems associated with sample preparation are greatly increased. Cornelius & Maduagwu (1986), using the coning and quartering method of sample preparation for palm kernels, found large variations in aflatoxin content in the various subsamples produced.

After comminution of the sample, an effective subsampling method is the use of rotary cascade dividers. Problems associated with milling and subsampling have been reduced also by the development of machines like the Dickens and Satterwhite mill, which is very efficient for the treatment of groundnuts. Currently, TDRI, in collaboration with two UK companies, is developing a subsampling mill capable of handling other commodities in addition to groundnuts.

The 'slurry technique' proposed by Velasco & Sheldon (1976) is a reliable method for the final reduction of sample size. In this procedure the subsample (1 kg) is slurried by blending with water in a high speed blender, thus distributing the aflatoxin uniformly through the mixture. Suitable aliquots are then taken from the slurry for analysis.

V. Problems associated with analysis

The choice of analytical procedure used for the determination of aflatoxin content can influence the result obtained. The analytical procedures used are continually being altered due to improved techniques, but the basic steps remain the same: extraction, lipid removal, clean-up, separation and quantification. The method used for the analysis of aflatoxins depends on whether either qualitative or quantitative estimation is required. For example, widely used qualitative techniques are the minicolumn method of Velasco (1972) and the BGYF (bright green-yellow fluorescence) method used for maize. A wide variety of techniques have been used to assess the concentration of aflatoxins, including thin-layer chromatography, high performance liquid chromatography, high performance thin-layer chromatography and the use of the fluorotoxinmeter. These procedures depend on the chromatographic separation of the aflatoxins, which are then estimated using their fluorescent properties under long-wave ultra-violet light.

VI. Statistical models for sampling plans

As discussed earlier, statistically-valid sampling plans are necessary to enable bulk consignments of commodities to be usefully analysed to determine their aflatoxin levels. Both the USDA and the TDRI plans are based on a negative binomial distribution of aflatoxin throughout the population of the commodity, which was proposed by Whitaker *et al.* (1974). Alternatively, the compound poisson-gamma distribution was suggested by Waibel (1977). Although this and the negative binomial distribution were shown by Knutti & Schlatter (1982) to differ markedly for single kernels, they approached each other for large samples as a consequence of the statistical law known as the Central Limit Theorem. As long as large sample sizes and high aflatoxin acceptance levels are used (as in USA) the correct choice of the underlying statistical distribution was not considered important. However, it was found to be critical if low aflatoxin levels are accepted and if the decision is based on small samples. Brown (1984) examined the suitability of the negative binomial distribution in describing aflatoxin levels in two varieties of peanuts, but found the model to be inadequate and suggested an alternative one.

None of the proposed statistical models have been found to be completely satisfactory and so we have chosen the three parameter Weibull model:

$$f(x) = \frac{\beta}{\eta}\left(\frac{x-\gamma}{\eta}\right)^{\beta-1} \exp\left[-\left(\frac{x-\gamma}{\eta}\right)^{\beta}\right] \qquad (\gamma < x < \infty)$$

where γ is the minimum aflatoxin level, β is the shape parameter and η is the scale parameter. These parameters can be estimated from the data by the method of maximum likelihood.

The reasons for choosing a Weibull distribution are:

(a) it is a distribution for a continuous random variable such as aflatoxin; and

(b) the three parameters in the Weibull distribution make it a very flexible distribution, which can be made to fit most unimodal data.

When looking at several sets of data it became apparent that there was not just one underlying distribution but three, in which the kernels contained:

(a) no aflatoxin;

(b) a low, and usually acceptable, level of aflatoxin; and

(c) an unacceptably high level of aflatoxin.

If x is the aflatoxin concentration in μg/kg, there is a proportion, p_1, of groundnuts with $x=0$. There is a proportion, p_2, corresponding to $0<x<120$, which can adequately be described by a Weibull distribution, and a proportion, p_3, where $x > 120$ described by a different Weibull distribution.

The proportion of each type of groundnut is estimated, as are the parameters of each of the two Weibull distributions. Values are then simulated from the combined distribution and these are compared with the raw data.

It is proposed that if, during testing, any of the third type of groundnuts is detected, the whole batch should be subjected to more detailed examination. It may be that p_1 is large and p_3 so small that the average aflatoxin level of the batch is less than the accepted upper limit.

The three parameter Weibull model was applied to analytical data obtained by us from three batches of groundnut cake and to the data published by Brown (1984) on Virginia Bunch peanuts, but excluding the zero observations. In the latter case there were 217 samples, of which 129 had no detectable aflatoxin, 60 had an aflatoxin content less than 120 μg/kg and 28 a content greater than 120 μg/kg. A dividing line of 120 μg/kg was taken by us for the study, but this value would vary from batch to batch. A single Weibull distribution gave a moderately good fit, but the two Weibull distributions gave very good fits.

References

Apelt, J.; Gerlach, M.; Jansen, H.D. (1985) Probenvorbereitung-Einfluss der Verfalrensstufen Teilen, Mischen und Zerkleinern auf das Analysenergebnis. *Die Mühle und Mischfuttertechnik* **8** (**122**), 96-99.

Brown, G.H. (1984) The distribution of total aflatoxin levels in composited samples of peanuts. *Food Technology in Australia* **36**, 128-130.

Coker, R.D.; Jewers, K.; Jones, B.D. (1985) The destruction of aflatoxin by ammonia: practical possibilities. *Tropical Science* **25**, 139-154.

Cornelius, J.; Maduagwu, E.N. (1986) The effects of aflatoxins on marketing of palm kernels from West Africa. In *Spoilage and mycotoxins of cereals and other stored products* (B. Flannigan, ed.), Biodeterioration Society Occasional Publication No. 2. Farnham Royal; Commonwealth Agricultural Bureaux International.

Cucullu, A.F.; Lee, L.S.; Mayne, R.Y.; Goldblatt, L.A. (1960) Determination of aflatoxins in individual peanuts and peanut sections. *Journal of the American Oil Chemists' Society* **43**, 89-92.

Dickens, J.W. (1977) Aflatoxin control programme for peanuts. *Journal of the American Oil Chemists' Society* **54**, 225A-228A.

Hansen, L.G. (1985) MAFF proposals for consolidating Feeding Stuff Regulations. *Feed Compounder* **5**, 28.

Jewers, K. (1982) Mycotoxins in food - the application of survey and quality control. *Royal Society of Health Journal* **102**, 114-118.

Kershaw, S.J. (1985) Aflatoxins in imported edible nuts: some data 1982-84. *Journal of Food Technology* **20**, 647-649.

Knutti, R.; Schlatter, C. (1982) Distribution of aflatoxin in whole peanut kernels - sampling plans for small samples. *Zeitschrift für Lebensmitteluntersuchung und -forschung* **174**, 122-128.

Velasco, J. (1972) Detection of aflatoxin using small columns of Florisil. *Journal of the American Oil Chemists' Society* **49**, 141-142.

Velasco, J.; Sheldon, L.M. (1976) Use of water slurries in aflatoxin analysis. *Journal of Agricultural and Food Chemistry* **24**, 86-88.

Velasco, J.; Whitaker, T.B. (1975) Sampling cottonseed lots for aflatoxin contamination. *Journal of the American Oil Chemists' Society* **52**, 191-195.

Waibel, J. (1977) Stichprobengrösse für die Bestimmung von Aflatoxin in Erdnüssen. *Deutsche Lebensmittel Rundschau* **73**, 353-357.

Whitaker, T.B.; Dickens, J.W.; Wiser, E.H.; Monroe, R.J. (1974) *Development of a method to evaluate sampling plans used to estimate aflatoxin concentration in lots of shelled peanuts.* Information Bulletin - Technical Reports, No. 10. Oxford; IUPAC Secretariat.

Spoilage and Mycotoxins of Cereals and other Stored Products.
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Efforts to Control the Levels of Aflatoxin in South-East Asian Maize

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I. Summary

Maize is an important crop in many South-East Asian countries and is utilised for internal consumption as in Indonesia and the Philippines, is an export commodity as in Thailand, or is an import commodity, for use as an animal feedstuff, as in Malaysia and Singapore. The high temperatures and humidities which are found in South-East Asia are ideally suited to mould infestation and aflatoxin formation if the crop is not properly dried and stored. It is not surprising, therefore, that aflatoxin contamination in maize produced and utilised in these countries sometimes exceeds acceptable levels. This paper reviews the results of surveys and surveillance programmes carried out in the region to determine the extent of aflatoxin contamination of maize and the stage in the production chain where contamination occurs. The results of studies carried out in Thailand on methods of controlling aflatoxin formation, particularly during the high-risk rainy season, are presented and the possible impact that they could have on future Thai maize production are discussed.

II. Introduction

Maize is an important crop in South-East Asia particularly in countries such as Thailand, Indonesia and the Philippines (Table 1). In many of these countries it is consumed as a staple food second only to rice. In some regions of Indonesia for example the daily *per capita* intake of maize can be as high as 300-500 g.

There is also a very extensive trade in maize in this area (Table 2). In Thailand, over 4 million tonnes of maize was produced in 1984, of which about 75%, worth over 400 million dollars, was exported. The Philippines utilises nearly all of its production internally and also imports significant quantities. Malaysia and Singapore also import large quantities of maize for use in animal feeds.

Unfortunately, the high temperatures and humidities which are found in South-East Asia are ideally suited to infestation by *Aspergillus flavus* with subsequent contamination by aflatoxin if maize is not properly dried and stored.

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Table 1. *The production of maize in the South-East Asian region*

Country	Area harvested (1000 ha)			Yield (kg/ha)			Production (1000 tonnes)		
	1982	1983	1984	1982	1983	1984	1982	1983	1984
Burma	141	136	200[a]	1466	1760	1799	206	239	360
Indonesia	2061	3018	2500	1569	1688	1600	3235	5095	4000[a]
Malaysia	8	14	14	1125	1429	1571	9	20	22
Philippines	3157	3365[a]	3405[a]	990	1018	999	3126	3425	3400[a]
Thailand	1306	1567	1660	2299	2267	2500	3002	3552	4150

(a) = estimated

Table 2. *The maize trade in some South-East Asian countries*

Country	Exports				Imports			
	Quantity (tonnes)		Value (1000 $)		Quantity (tonnes)		Value (1000 $)	
	1983	1984	1983	1984	1983	1984	1983	1984
Burma	30300	28000[a]	3600	2800[a]	–	–	–	–
Indonesia	17936	159853	2555	21400[a]	28189	59386	5250	9660
Malaysia	2035	–	247	–	777533	953000[a]	115664	148000
Philippines	35	–	54	–	528441	182441	70747	28859
Singapore	166602	152377	26533	24468	564043	469931	77915	67868
Thailand	2630045	3116742	364641	426552	815	500[a]	720	450

[a] = estimated

Sources: FAO Production Yearbook, 1984, and FAO Trade Yearbook, 1984

III. The Problem of Aflatoxin in Maize

Aflatoxin contamination of maize has been shown to occur in virtually all the producing areas of the world and it has been shown that contamination can occur either pre- or post-harvest. The problem has been studied extensively in the USA where pre-harvest contamination, in particular, is a serious problem. Although not all the steps in the pre-harvest infection process are understood it has been demonstrated that *A. flavus* can colonise the maize silks and then invade the developing kernels (Payne 1983) and that the fungus can grow from the external silks into the ear within four days. Although insects seem to play an important role in the infection process, they may be more important in providing infection sites by damaging the kernels and in spreading the fungus within the ear than in bringing in the primary inoculum (Payne 1983). The major factor leading to increased susceptibility to contamination is plant stress, in particular moisture stress, but other factors such as temperature, mechanical damage and variety are also important (Lillehoj 1983). For example in one study (Zuber *et al.* 1983) it was shown the mean aflatoxin level at harvest in four open-pollinated varieties of maize was significantly higher than that in grain from four adapted maize hybrids. Unfortunately, although there is varietal variation in susceptibility to *A. flavus* infection no varieties have yet been found which are resistant to contamination.

Post-harvest contamination of maize, as with other commodities, is caused by slow or inadequate drying after harvest or poor storage. Ideally, provided maize is dried quickly to below 12% moisture and stored properly (Anon. 1981) aflatoxin contamination should not occur.

IV. The Occurrence of Aflatoxin in Maize of South-East Asia

1. Philippines

The potential for the contamination of commodities by aflatoxin is very high in the Philippines because of the prevailing high temperatures (annual mean 27°C) and humidities (annual mean 80%), particularly since agricultural practices both pre- and post-harvest tend to be inadequate and inefficient.

Early studies indicated that maize and groundnut samples contained high levels of aflatoxin. Other work also established a link between the consumption of aflatoxin-contaminated maize and the occurrence of primary liver cancer in Filipinos.

Although aflatoxin contamination seems to be essentially a post-harvest problem in the Philippines, *Aspergillus flavus* and *A. parasiticus* were found to infect maize in the field, particularly when the ears were damaged, or in insect-infested, over-matured grains (Ilag, 1973). Post-harvest infection was highest in maize samples that were dried and/or stored after shelling, lower in cobs without husk and lowest in maize cobs with intact husks. The degree of infection was directly related to the amount of *A. flavus* inoculum in the air which was least in the fields, higher in the drying areas and highest in the warehouses.

2. Indonesia

Maize is used as a staple food throughout Indonesia and again, because of the warm humid climate, infestation by *A. flavus* is a problem.

A limited survey on aflatoxin levels in maize purchased at the local markets in Bogor was carried out between November 1978 and October 1979 (Hetzel & Sutkino 1979): 22 samples of local maize were analysed, which had a mean aflatoxin B_1 content of 164 μg/kg with a range of aflatoxin levels of 21-371 μg/kg. The mean aflatoxin B_1 content of 24 samples of imported Thai maize was 45 μg/kg with a range of aflatoxin levels of 2-139 μg/kg.

A more recent survey was carried out jointly by the National Logistics Agency (BULOG) and the Tropical Development and Research Institute (TDRI) (Sintha *et al.* 1983). Samples of maize were collected from five administrative areas or DOLOGS of East Java, Lampung, North Sulawesi, South Sulawesi and West Nusa Tenggara. Particular attention was given to the sampling procedures so that the samples collected, about 12 per DOLOG, would be representative. For example, in order to obtain a 10 kg representative sample from 20 tonne batches (200 sacks) entering or leaving the Sub-DOLOG store, 100 x 100 g subsamples were taken from every other sack with a spear sampler. The results of this survey are given in Table 3.

Table 3. *Aflatoxin contents of maize samples from 5 provinces of Indonesia*

Province	No. of samples	Percentage positive	Total aflatoxin content (μg/kg)	
			Mean	Highest
East Java	15	100	149	390
Lampung	12	92	144	350
North Sulawesi	12	100	464	790
South Sulawesi	12	100	108	250
West Nusa Tenggara	12	83	180	1140

The survey showed a 95% incidence of aflatoxin contamination of maize, with 86% of the samples containing more than 30 μg/kg total aflatoxins. The mean aflatoxin contentration of the 63 samples was 218 μg/kg. If only the samples of processed or semi-processed maize were included the mean of these 56 samples was 241 μg/kg. There was circumstantial evidence indicating that the drying stage was likely to be the primary cause, since the seven samples of unprocessed material, i.e. maize sampled within 1-2 days of harvest, had a mean total aflatoxin content of only 34 μg/kg.

The use of the bright-greenish yellow fluorescence (BGYF) test (Shotwell & Hesseltine 1981), as a rapid method of examining samples for quality control purposes, was also examined during this study. Although field testing, using whole kernels, gave too many false negative results to be of use, laboratory BGYF testing on ground material gave promising results, as can be seen from Table 4.

Table 4. *Number of samples and mean aflatoxin level for each BGYF class*

BGYF classification	No. of samples	Mean aflatoxin content (μg/kg)
low	12	34
medium	26	160
high	12	288
very high	6	360
extremely high	5	542

This initial study indicated that samples with low or high aflatoxin contents, i.e. <30 or >500 μg/kg, could be reliably identified but for those with intermediate levels the method was not so good.

3. Thailand

An aflatoxin survey was carried out in Thailand between September 1967 and July 1969 of validated market food samples collected in a large number of towns and villages (Shank *et al.* 1972). Over 2000 samples were collected in all, representing more than 170 different kinds of foodstuffs, the largest single categories being grains, beans and seeds, the major constituents of the diet. Of the 62 samples of maize analysed, 35% were found to be contaminated, the mean total aflatoxin content of the contaminated samples being 400 μg/kg.

In order to minimise the problem of aflatoxin contamination of maize, a programme organised by the Department of Agriculture was initiated in 1980 to survey maize at different stages of growth, harvesting, handling and storage for aflatoxin contamination (Prisnar *et al.* 1981). In 1980, samples of maize collected from Phetchaboon Province pre-harvest (40 samples) and after storage on the farm for one or two months (16 and 8 samples, respectively), were all found to contain less than 20 μg/kg of aflatoxin. During 1981-2, of 50 samples of maize collected from five provinces pre-harvest only one sample contained aflatoxin at a level of 20 μg/kg. These results indicate that pre-harvest aflatoxin contamination of maize in Thailand is unlikely to be a problem.

Further results from this study have since been published (Prisnar *et al.* 1983) and these confirm the initial results as far as pre-harvest aflatoxin contamination is concerned (see Table 5). However, the analytical results for 114 post-harvest samples taken at various points in the marketing chain showed that the incidence of contaminated samples was 77% (Table 5).

Table 5. *The incidence of aflatoxin in maize at different points in the marketing chain*

Sampling point	No. of samples	Percentage of positive samples	Aflatoxin content (μg/kg) mean (min–max)	Moisture content (%) mean (min–max)
Preharvest	130	4.6	0.6 (0–33)	23.1 (15.0–28.8)
On Farm				
After 1 month	16	25.0	7 (0–120)	17.6 (13.4–24.7)
After 2 months	8	62.5	21 (0–75)	18.7 (13.4–24.7)
Middlemen's Store				
After 1 month	22	86.4	196 (0–750)	19.2 (14.0–29.3)
After 3 months	28	78.6	104 (0–435)	16.2 (11.5–23.8)
Silos	40	97.5	93 (0–362)	14.0 (12.1–18.2)

These figures for aflatoxin contamination in maize in Thailand are not surprising since the main maize crop is harvested during the monsoon season, which makes any sun drying of the crop extremely difficult. Therefore, in recent years, because the quality of Thai maize has suffered because of aflatoxin contamination, the effect on the economy has been substantial due to lost export markets and discounted prices. It was for this reason that, following initial discussions early in 1984, a joint Thai-UK project was established to investigate the incidence of aflatoxin in Thai maize and to determine whether levels could be controlled by effective drying.

V. The Thai-UK Maize Aflatoxin Project

During the first phase of this project, which was carried out in Lopburi Province and was completed in 1984, mechanical drying was carried out at three different stages in the marketing chain using various drying systems and their effectiveness was compared with the traditional sun-drying technique.

The results of the first stage of the project are detailed in Table 6. These indicate that, although aflatoxin contamination was low at harvest, a significant increase in aflatoxin levels occurred during

longer-term farm storage and temporary storage at the local merchants. However, mechanical drying was capable of preventing any further increase in aflatoxin levels provided that the drying period did not exceed 48 h. It was also shown that maize dried either mechanically or by sun-drying to a moisture content of 14% could be stored for at least two months without any increase in aflatoxin over its initial level, provided that the storage conditions were good.

Table 6. *Results of Thai-UK maize project – phase 1*

Sampling point	No. of samples	Mean total aflatoxin content (μg/kg)
At harvest	0	0
Farm storage within 2 to 14 days of harvest	13	21
Farm storage after 2 months	10	76
Local merchants within 24 hr of shelling	10	45
Incoming to silos in Bangkok	30	218
After drying at silos	19	187
Before drying[a] at local merchants	10	71
After 2-stage drying[a] over 5 days at local merchants	10	126
Before drying[b] at regional merchants	10	176
After 1 stage drying[b] over 48 hrs at regional merchants	10	181

(a) This trial (10 replicates) was conducted on a 10 tonne scale using cobs which were processed within 1–4 weeks of harvest. The cob drying system comprised a storage bin and a Lister ST2 moisture extraction unit (MEU) which reduced the moisture content to 18–20%. After shelling the maize was dried down to 14% moisture content using a Lister LT1 MEU.
(b) This trial (10 replicates) was carried out on a 12–23 tonne scale using a shelled maize and a drying system based on a Lister ST3 MEU. The maize was dried to a moisture content of 14% over a period of 48 hrs.

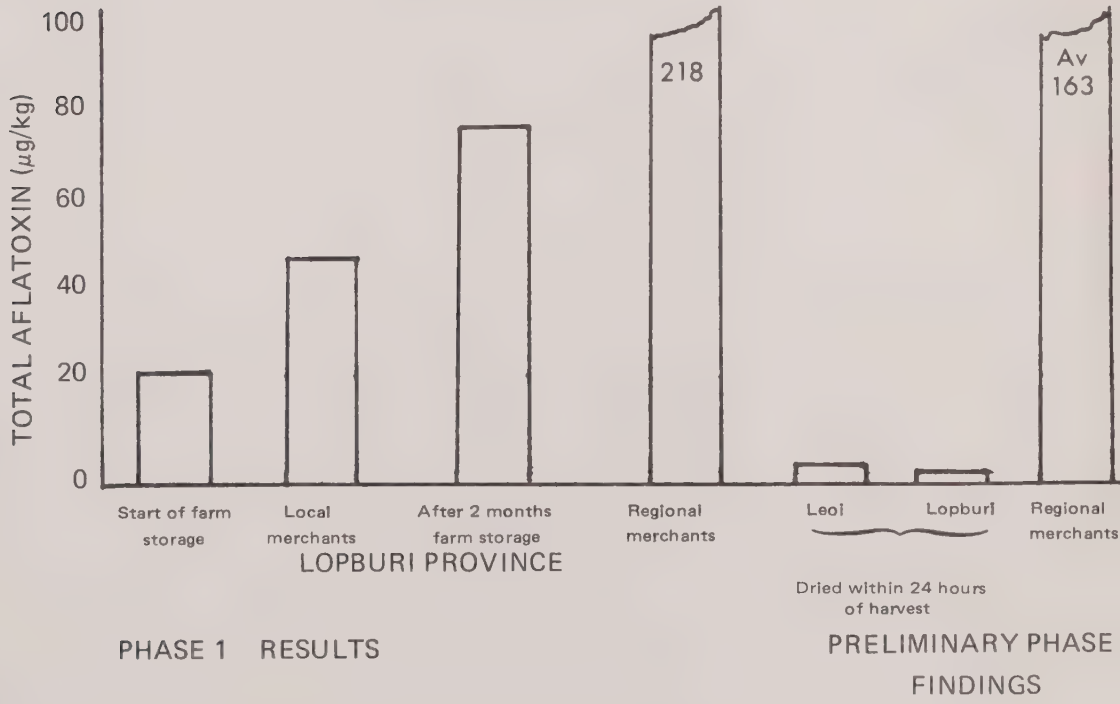

Fig. 1. Comparison of phase 1 results with preliminary phase 2 findings

The initial phase of this project indicated that control measures were required immediately after harvest and that drying to 14% moisture content within 48 h could prevent aflatoxin contamination. Ideally the drying should be carried out at one site so as to avoid any transportation and temporary storage of the wet maize, which it has been found can lead to high levels of aflatoxin contamination.

In phase 2 of the project, which started in 1985 further work was carried out to confirm the findings of phase 1 and to relate them to a further maize producing area, viz. Leoi. A programme of field trials was also carried out on the designing, testing and evaluation of specific drying systems for farmers cooperatives and primary merchants. Storage trials were also carried out to determine whether maize with a moisture content of greater than 14% could be safely stored for a short period of time, without an unacceptable increase in aflatoxin, before final drying.

The preliminary findings from this second stage of the project compared to the results from the 1984 trials are shown in Fig. 1.

It is anticipated that the complete findings from phase 2 of this project will be available in the near future.

VI. Conclusion

Aflatoxin contamination of maize, either pre- or post-harvest, has been shown to be a problem in most areas of the world where maize is produced. Studies in South-East Asia have demonstrated that contamination of maize by aflatoxin is a recurrent problem, due to the high humidities and temperatures that occur in the region. However, infestation with *A. flavus* and aflatoxin contamination appears primarily to occur post-harvest and is mainly caused because of slow or inadequate drying. This is because natural drying of the maize crop is extremely difficult in most countries in the area since it is harvested in the rainy season.

In recent years Thailand, which is a large exporter of maize, has suffered because of high aflatoxin levels. Export markets have been lost, prices discounted and the economy has suffered. Because of the seriousness of the problem a joint Thai-UK project was initiated in 1984 in order to investigate the incidence of aflatoxin in Thai maize and to determine whether effective control measures could be introduced.

The study has shown that a good quality product, with a low aflatoxin content, could be produced by drying the maize to a moisture content of 14% or less within 48 h of harvest. Storage of this material under good conditions for up to two months did not give rise to an increase in aflatoxin level.

References

Anon. (1981) Storage of cereals and pulses - Part 1: General considerations in keeping cereals. *International Standard, ISO 6322/1.*

Hetzel, J.; Sutikno, I. (1979) A report of aflatoxin contamination in local and imported corn and soybean meal in West Java, Indonesia. *Proceedings of the International Symposium on Microbial Aspects of Food Storage, Processing and Fermentation in Tropical Asia, Bogor, Indonesia, December 1979.*

Ilag, L.L.; Garcia, R.P.; Benigno, E. (1983) Mycotoxin contamination in agricultural commodities. *Proceedings of the National Post Harvest Institute for Food & Nutrition/FAO Technical Symposium on the Advances in Food Protection and Preservation, Manila, October 1983.*

Lillehoj, E.B. (1983) Effect of environmental and cultural factors on aflatoxin contamination corn kernels. In *Aflatoxin and Aspergillus flavus in Corn* Southern Cooperative Series Bulletin 279 (U.L. Diener; R.L. Asquith; J.W. Dickens, eds), 27-34. Alabama; Alabama Agricultural Research Station/Auburn University.

Payne, G.A. (1983) Nature of field infection of corn by *Aspergillus flavus.* In *Aflatoxin and Aspergillus flavus in Corn* Southern Cooperative Series Bulletin 279 (U.L. Diener,; R.L. Asquith; J.W. Dickens, eds), 16-19.Alabama; Alabama Agricultural Research Station/Auburn University.

Prisnar, H.; Arunsri, W.-U.; Dara, B. (1981) Incidence of aflatoxin in pre- and post-harvested corn. *Thailand National Corn and Sorghum Programme, Annual Report*, 129-133.

Prisnar, S.; Arunsri, W.-U.; Prawat, T.; Dara, B. (1983) Incidence of aflatoxin in corn. *Proceedings of the Sixth Annual Workshop on Grain Post-Harvest Technology, Bogor, Indonesia* (N.C. Teter; R.L. Semple; A.S. Frio, eds), 341-348.

Shank, R.C.; Wogan, G.N.; Gibson, J.B.; Nondasuta, A. (1972) Dietary aflatoxins and human liver cancer. II. Aflatoxin in market foods and foodstuffs in Thailand and Hong Kong. *Food and Cosmetics Toxicology* **10**, 61-69.

Shotwell, O.L.; Hesseltine, C.W. (1981) Use of bright greenish yellow fluorescence as a presumptive test for aflatoxin in corn. *Cereal Chemistry* **58**, 124-127.

Sintha, W.; Nagler, M.J.; Kulinar, S.; Iteng, Z. (1983) Aflatoxin survey of corn in Indonesia. *Proceedings of the Sixth Annual Workshop on Grain Post-Harvest Technology, Bogor, Indonesia* (N.C. Teter; R.L. Semple; A.S. Frio, eds), 329-340.

Zuber, M.S.; Darrah, L.L.; Lillehoj, E.B.; Josephson, L.M.; Manwiller, A.; Scott, G.E.; Dudauskas, R.T.; Horner, E.S.; Widstrom, N.W.; Thompson, D.L.; Blockholt, A.J.; Brewbaker, J.L. (1983) Comparison of open pollinated maize varieties and hybrids for preharvest aflatoxin contamination in the United States. *Plant Disease* **67**, 185-187.

Spoilage and Mycotoxins of Cereals and other Stored Products.
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The Possible Effect of Aflatoxin Contamination on the Marketing of Palm Kernels from West Africa

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I. Summary

Palm kernel production in West Africa and the methods used to produce the kernels are described. Exports from West Africa and imports into the EC of palm kernels and cake, and their proportions of world trade, are provided for the years 1982-1984.

The proposed changes by the EC to the maximum permitted levels of aflatoxin in imported agricultural products could affect the exports of palm kernels and cake from West Africa. Information is given which shows that *Asperigillus flavus* is a major contaminant of palm kernels. Preliminary studies suggest that kernels produced by small farmers could contain higher levels of aflatoxin than those produced in oil palm estates.

Distribution studies on aflatoxin in samples drawn from two 1-tonne batches of palm kernels are described. These show that specially constructed sampling plans are required if the levels of aflatoxin in large batches are to be predicted with a high degree of confidence. Data is given on the efficiency of coning and quartering. The technique has been shown to be inappropriate for producing a representative sub-sample for aflatoxin analysis.

II. Introduction

Palm kernels are derived mainly from the West African oil palm *Elaeis guineensis* Jacq. The palm is believed to have originated in the swamps of West Africa where, even today, the greater part of the industry is dependent upon the exploitation of semi-wild groves which start from the 10-11°N parallel on the West Coast, southward and around the coast through the southern latitudes of Sierra Leone, Liberia, The Ivory Coast, Ghana, Togoland, Dahomey, Nigeria, Cameroons and into the equatorial regions of Brazzaville and Zaire down to about 10-11°S. Isolated areas north and south of the main belt, and also in East Africa, are limited to places where the rainfall is sufficient. A considerable proportion of the oil palm in Zaire and the Ivory Coast is grown on plantations and in

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places where the oil palm has been introduced into the Far East, India, Sri Lanka, and Central and South America, the industry is entirely plantation based.

III. Description

The fruit of the oil palm grows in bunches which weigh from 4 to 20 kg and contain 200-2000 individual fruits. Each is a sessile drupe, which varies in shape from nearly spherical to ovoid or elongated and bulging somewhat at the distal end, and from 3g to over 30g in weight. The fibrous mesocarp yields some 50% by weight of palm oil and the kernel, usually enclosed in a hard woody shell, is the source of palm kernel oil which differs greatly from palm oil in appearance, characteristics and compostion. Grown under plantation conditions, oil palm varieties are selected for high mesocarp content and palm oil is the major product, but under semi-wild conditions in West Africa, the mesocarp is frequently thin and yields relatively little oil. Palm kernels then become the major product.

IV. Processing

In West Africa, under primitive conditions and in cases where a mesocarp oil of low free fatty acid (ffa) is not required, the fruit may be loosened from the bunch by allowing the heaped-up fruit to ferment. Where a low ffa is desired, the lipase in the fruit must be inactivated, and this is carried out by boiling in water the fruit which has been stripped from the bunch, or better, by boiling the fruit on the bunches. The hot stripped fruit is then digested by pounding with a pole or pestle in some kind of mortar which may be a hollowed-out tree trunk or a hole in the ground, but is preferably a mortar made of concrete. The palm oil is expressed from the digested fruit, which still contains the whole nut, either by primitive means such as stamping with the foot, squeezing in the hand, boiling in water and skimming off the oil or, more effectively, by using small presses.

The nuts are separated from fibre after extraction of the palm oil from the mesocarp, allowed to dry and cracked, either by hand by the women and children in the villages or mechanically in a small machine. Under plantation conditions, fruit is sterilized on the bunch, using high pressure (about 2.8 kg/cm^2 or 40 psi) steam, and the oil is extracted from the mechanically-stripped and digested fruit, either by means of hydraulic presses or, more usually nowadays, by means of continuous screw presses.

The residue contains the nuts which must be separated from the mesocarp fibre. The latter is used as a fuel for the mill boilers and the former are dried, cracked and the kernels (relative density, about 1.07) separated from the shells (relative density, 1.3-1.4) either by flotation in a clay bath or by means of hydrocyclones (Cornelius 1977)

V. Handling and Storage

Fresh unsterilized kernels have a moisture content of about 22% and need to be dried as rapidly as possible to prevent fungal growth. Out of nine species of fungi isolated from Nigerian palm kernels, seven were found to be lipolytic, including *Aspergillus flavus*, of which certain strains are aflatoxin producing (see Table 1).

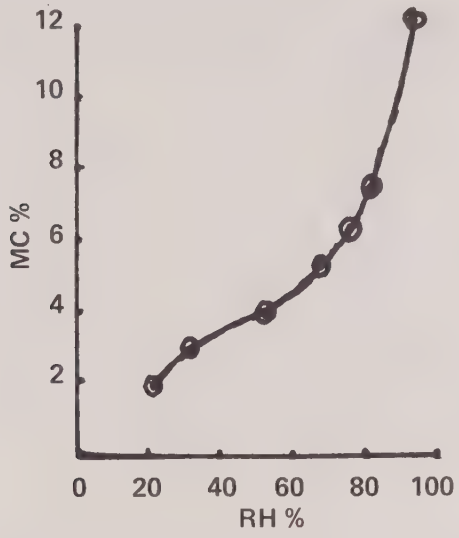
Table 1. *Incidence of fungi on palm kernels and their lipolytic action.*

Species	Occurrence (%)	Lipolytic activity
Aspergillus flavus	35	++
Aspergillus fumigatus	20	++
Paecilomyces variotii	10	+
Syncephalastrum racemosum	10	++
Aspergillus chevalieri	5	+
Aspergillus niger	5	++
Aspergillus terreus	5	−
Penicillium steckii	5	+
Penicillium citrinum	5	−

++ = Actively lipolytic; + = Lipolytic; − = Non-lipolytic (Coursey et al. 1963).

VI. Production

The production of palm kernels in West Africa, shown in Table 2, indicates that it has totalled around 650,000 tonnes over the last three years, that is from 1982 to 1984, but the percentage of total West African production has been falling, largely due to substantial increases from the Far East, especially Malyasia.



(Somade, 1955)

Fig. 1. Moisture equilibrium of palm kernels

Ideally, palm kernels should be dried to about 6 - 7% moisture content before bagging, since a final moisture content higher than 7% would permit the development of fungal growth on the kernels during storage. However, whatever moisture content sterilized kernels have initially on storage, they will eventually come to equilibrium with the prevailing relative humidity. The curve in Fig. 1,based upon the data of Somade (1955), shows the variation in the mean equilibrium moisture content for palm kernels stored in Nigeria at various relative humidities for up to 35 days at 24-31°C. The average relative humidities for the palm kernel producing and handling area of Nigeria is approximately 82% for the four months, June to September, and 76% from October to May, which results in moisture contents at the equilibrium of approximately 7.7% and 6.4% respectively. It has been suggested that the maximum permissible figure for safe storage of oilseeds generally is 14% of moisture calculated on the non-oleaginous portion of the seed. With an oil content of 52%, on a dry basis this would amount to 7.25% of moisture in the whole seed, or somewhat lower than the mean equilibrium moisture content experienced for 4 months of the year in the palm kernel area of Nigeria (Cornelius 1966).

VII. Exports

Table 3 shows exports from West Africa of palm kernels at about 70,000 tonnes for 1982 about 86,000 tonnes in 1984 but 105,000 tonnes in 1983 due to a substantial increase in exports from Nigeria in that year. Exports of cake from West Africa fell from 77,000 tonnes in 1982 to 50,000 tonnes in 1984, doubtless due to increased domestic usage.

It will be noted that whereas kernel exports from West Africa comprise well over 50% of total world export, palm kernel cake exports are less than 10% of world exports, reflecting increasing exports of palm kernel from the Far East, which amounted to nearly 600,000 tonnes in 1984.

VIII. Imports

Imports of palm kernels and palm kernel cake (Table 4) into EC countries over the past 3 years indicate a drop in kernel imports from 100,000 tonnes to 63,000 tonnes, but an increase in total value in US dollars from $29 million to $32 million and a rise in cake imports from 489,000 tonnes valued at US $80 million in 1982 to 666,000 tonnes valued at US $116 million in 1983, with a slight decrease (625,000 tonnes valued at US $90 million) in 1984. In 1984, the EC imported 64% of the world total imports of kernels and 98% of cake of total value US $41 million. Thus palm kernels and palm kernel cake are important commodities in EC trade with West Africa.

Table 2. *Palm kernel production*

	'000 tonnes			
	1974–76	1982	1983	1984
World total	1,387	2,255	2,136	2,452
West Africa				
Benin	83	75F	75F	75F
Cameroon	39	52F	45F	47F
Gambia	1	2F	2F	2F
Ghana	33	30F	30F	30F
Guinea	35	35F	35F	35F
Guinea Bissau	8	10*	6*	11*
Ivory Coast	37	34	28	31*
Liberia	14	8	8F	8F
Nigeria	302	355F	360F	365F
Senegal	6	6F	6F	6F
Sierra Leone	47	30F	30F	30F
Togo	14	13F	15F	15F
Total West Africa	619	650	640	655
West Africa as % of world total	45	29	30	27

Source: FAO Production Yearbook
F = FAO estimate
* = non official data

Table 3. *Palm kernel and palm kernel cake exports*

	'000 tonnes					
	1982		1983		1984	
	Kernels	Cake	Kernels	Cake	Kernels	C
World total	110.1	619.2	141.1	720.1	121.9	6
West Africa						
Cameroon	2.3	2.4*	6.4	1.7	15.0	
Guinea	12.3		7.0*		6.0*	
Guinea Bissau	7.1		5.2		6.9	
Ivory Coast	6.8	12.5	4.5	16.6	16.0*	
Benin		12.4*		12.4*		
Liberia		2.5*		2.2*		
Nigeria	29.0	42.6*	73.0*	31.5*	28.0*	
Senegal		2.8*		2.0*		
Sierra Leone	6.5	1.4*	8.3	2.5*	12.9	
Togo	6.5		0.5	2.5	1.0*	
Total West Africa	70.5	76.6	104.9	71.4	85.8	
West Africa as % of world total	63.7	12.4	74.3	9.9	70.4	

Source: FAO Trade Yearbook
* = non official data

IX. Aflatoxin limits applied in the European Community (EC)

The EC adopted a directive in 1976 restricting the amount of aflatoxin permitted in animal feedstuffs to levels not exceeding 50 μg/kg (Jewers 1982). More recently, the European Parliament has endorsed a Commission proposal that maximum permitted levels of aflatoxin in raw materials used in animal feeds should be fixed, and where exceeded, should not be imported or put to further use. It proposed 200 μg/kg at 12% moisture (not 300 μg/kg as suggested by the Commission) with a reduction to 100 μg/kg as soon as technically feasible (Hanson 1985). The British Government is unlikely to increase the level it has set for imported groundnut cake (50 μg/kg) to the level proposed as this could lead to unacceptable levels of aflatoxin M_1 in milk.

Table 4. *Palm kernel and palm kernel cake imports into EC countries*

	'000 tonnes					
	1982		1983		1984	
	Kernels	Cake	Kernels	Cake	Kernels	Cake
Total world imports	122.3	617.6	137.4	734.3	98.1	637.9
EC countries						
Belgium/Lux		4.2		8.8		7.6
Denmark	24.0	53.1	22.1*	45.3	17.9	22.5
France	1.6	5.4	0.9	4.4	0.2	3.4
Germany FR		223.7	0.1	281.8		249.4
Greece				0.1		
Ireland		0.6				
Netherlands	10.5	178.5	3.7	316.0	1.1	330.3
UK	66.8	23.8	66.3	9.2	43.5	11.9
Total EC	102.9	489.3	93.1	665.6	62.7	625.1
EC as % of world total	84.1	79.2	67.7	90.6	63.9	98.0

Source: FAO Trade Yearbook
* = non official data

X. Analytical methodology

The oily nature of palm kernels can cause problems in the determination of their aflatoxin content. Freshly ground palm kernels can be sub-divided, but the particles adhere after standing for a short time making sub-division difficult. An aqueous slurry technique (Velasco & Morris 1976) is suitable for preparing an analytical sample (palm kernel: water; 1:1.5-3.0). A modified Romer technique can be used to extract aflatoxin from palm kernel products, but a bonded phase clean-up method developed at TDRI (Coker & Tomlins 1986), coupled with HPTLC, appears to substantially reduce the time required for analysis as well as improving the efficiency of the method.

XI. Preliminary studies

In view of previous reports of aflatoxin contamination of palm kernel products (Abalaka 1984, Bassler 1981, Buckle 1983, Dietrich & Hoffmann 1978), an investigation was undertaken to determine whether aflatoxin contamination could affect the trade in palm kernel products.

Table 5 shows the aflatoxin contents of single palm kernel samples taken from large consignments imported into the United Kingdom from 9 West African countries. A wide variation, from less than 3 to 1,610 μg/kg, in total aflatoxin content was revealed (Cornelius 1984). Six of the samples have aflatoxin contents in excess of 100 μg/kg, equivalent to over 200 μg/kg in the oil-free meal derived from them, which exceed the new levels recommended by the European Parliament. The data also suggests that palm kernels supplied by oil palm estates could contain significantly less aflatoxin than those produced by small farmers.

These findings prompted visits to three major West African palm kernel producing countries at the end of their dry seasons (February/March) to collect samples for analysis. Up to 20 kg samples were collected from large batches of palm kernels and palm kernel cake, and these were coned and quartered to produce 1 kg sub-samples for analysis. Analysis confirmed the preliminary findings: 4 out of 7 samples from country A (small farmers) had aflatoxin contents in excess of 100 μg/kg, whilst out of 15 samples collected in country B (estates), 4 samples contained between 1.7 and 10 μg/kg and the other 11 samples had no aflatoxin, and the 24 samples collected in country C (small farmers/estates) had aflatoxin levels between 0 (1 sample) and 35 μg/kg. However, 3 out of 10 samples collected in the middle of the rainy season in June from one particular country (C) contained over 100 μg/kg and two samples over 50 μg/kg. This suggested that seasonal factors might affect aflatoxin levels.

XII. Distribution of aflatoxin in 1-tonne batches of palm kernels

Figs. 2 and 3 show the distribution of aflatoxin in samples drawn from two 1-tonne batches of palm kernels. Approximatly 8 kg were drawn from the first batch in a random manner; the sample consisted of 18 approximately 400 g sub-samples. The aflatoxin content of the 8 kg sample has an asymmetric distribution (left-skewed; Fig. 2) with a mean of 448 μg/kg. In the second study, a 26.8

Table 5. *Aflatoxin contents of single palm kernel samples (0.5–1 kg) from 9 West African countries*

Sample	Aflatoxin (μg/kg)					Source
	B_1	B_2	G_1	G_2	Total	
1	ND	ND	ND	ND	0	Plantation
2	6	ND	ND	ND	6	
3	ND	13	ND	ND	13	
4	74	38	ND	ND	112	Plantation, small/farmer
5	112	46	ND	ND	158	Small farmer
6	150	18	ND	ND	168	
7	120	60	ND	10	190	
8	124	30	108	24	286	
9	1,488	122	ND	ND	1,610	

ND = not detected; detected limit, 3 μg/kg
Cornelius (1984)

kg sample consisting of 15 x 1.5-2.3 sub-samples was obtained from a second 1-tonne batch in a random manner. The aflatoxin distribution in this sample was very much less skewed (Fig. 3); the mean of this sample was 135 μg/kg. It is clear from these studies that a large sample is required if the level of aflatoxin in the batch is to be predicted with any degree of confidence, and that further work is required before a sampling plan can be elaborated for palm kernels.

XIII. The effect of coning and quartering on aflatoxin distribution

In view of the distribution of aflatoxin in the 26.8 kg sample, the effect of the coning and quartering on aflatoxin distribution was undertaken. Fig. 4 shows the way that aflatoxin is distributed in 16 sub-samples obtained by coning and quartering a 19.83 kg sample of palm kernels drawn from a sack of palm kernels.

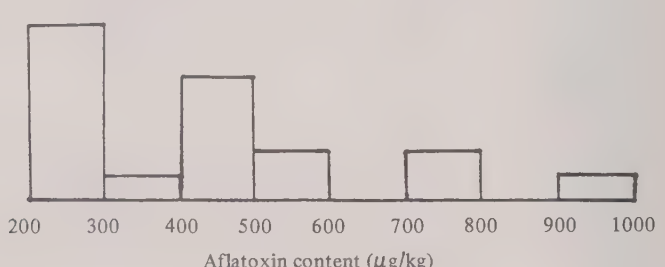


Fig. 2. Distribution of aflatoxin in a 1 tonne batch of West African palm kernels (18 x 400 g samples: $\bar{X}$ = 448)

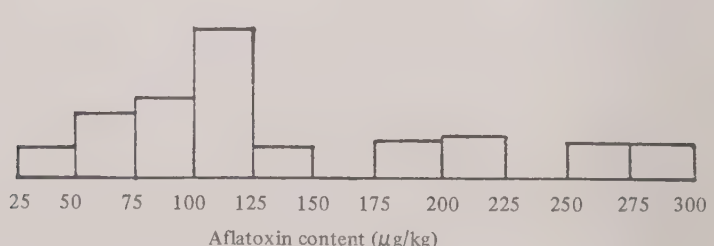


Fig. 3. Distribution of aflatoxin in a 1 tonne batch of West African palm kernels (15 x 1.5-2.3 kg samples:$\bar{X}$ = 135)

The distribution is slightly skewed to the right, with a mean of 245 μg/kg. It is clear that the technique is not suitable for obtaining a representative sub-sample for aflatoxin analysis, and that care must be taken in interpreting the data recorded in Table 5 because of the large variance obtained when analysing small samples.

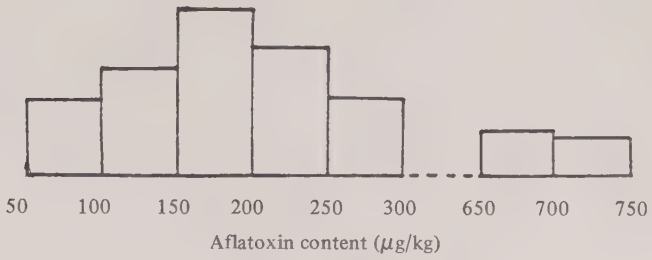


Fig. 4. Distribution of aflatoxin in a 19.83 kg sample of palm kernels separated into 16 portions by coning and quartering

XIV. Conclusions

Palm kernels and palm kernel cake are important sources of foreign exchange for a number of West African countries. Aflatoxin contamination of both commodities does occur, and evidence suggests that it could be more of a problem in kernels produced by small farmers. The European Parliament's proposal that maximum permitted levels of aflatoxin in raw materials used in animal feeds should be fixed at 200 μg/kg at 12% moisture could affect exports of kernels and cake to the EC. A suitable sampling plan for both commodities is urgently required if remedial action is to be taken to control the levels of aflatoxin in palm kernel products.

References

Abalaka, J.A. (1984) Aflatoxin distribution in edible oil-extracting plants and in poultry feedmills. *Food and Chemical Toxicology* **22**, 461-463.

Bassler, R. Experiences with the examination of feeds and some oil seeds for aflatoxin. *Qualitas Plantarum, Plant Foods for Human Nutrition* **30**, 271-282.

Buckle, A.E. (1983) The occurrence of mycotoxins in cereals and animal feedstuffs. *Veterinary Research Communications* **7**, 171-186.

Coker, R.D., Tomlins, K. (1986) Private communication.

Cornelius, J.A. (1966) Some technical aspects influencing the quality of palm kernels. *Journal of the Science of Food and Agriculture* **17**, 57-61.

Cornelius, J.A. (1977) Palm oil and palm kernel oil. *Progress in the Chemistry of Fats and other Lipids* **15**, 5-27.

Cornelius, J.A . (1984) News item: aflatoxins in palm kernels. *Oil Palm News* **28**, 3-5.

Coursey, D.G., Simmons, E.A. Sheridan, A. (1963) Studies on the quality of Nigerian palm kernels. *Journal of the West African Science Association* **8**, 18-23.

Dietrich, H., Hoffmann, G. (1978) The aflatoxin content of oilseed residues and the reliability of its estimation. *Landwirtschaftliche Forschung* **31**, 19-25.

Hanson, L.G. (1985) MAFF proposals for consolidating Feeding Stuffs Regulations. *The Feed.Compounder* **5**, 28.

Jewers, K. (1982) Mycotoxins in food - the application of survey and quality control. *Royal Society of Health Journal* **102**, 114-118.

Somade, B. (1955) Moisture equilibrium of palm kernels. *Journal of the Science of Food and Agriculture* **6**, 426-427.

Velasco, J., Morris, S.L., (1976) Use of water slurries in aflatoxin analysis. *Journal of Agricultural and Food Chemistry* **24**, 86-88.

Spoilage and Mycotoxins of Cereals and other Stored Products.
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The Treatment of Aflatoxin Contaminated Commodities

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I. Summary

Aflatoxin-contaminated food and feeds may be decontaminated by segregation or degradation methods. The former involve the removal of aflatoxin from the contaminated batch whilst the latter facilitate the conversion of aflatoxin to a non-toxic derivative. Segregation methods include quality control procedures and solvent extraction techniques whereas the degradation of aflatoxin may proceed by physical, chemical and biological action. The utilisation of ammonia gas has been extensively investigated and commercial facilities exist for the decontamination of groundnut cake by ammoniation.

II. Introduction

The aflatoxins are highly toxic fungal metabolites, produced by the moulds *Aspergillus flavus* and *A. parasiticus*, which occur in a wide variety of foods and feeds in tropical environments. The ingestion of these toxins can have a deleterious effect on the health and productivity of man and animals alike, and can cause death when consumed in sufficiently high quantities (Coker 1979). Consequently, it is highly desirable that the incidence of these compounds in foods and feeds should be kept to a minimum. This may be partly achieved by the implementation of, for example, efficient horticultural, harvesting, drying, transportation and storage practices. However, the ubiquitous nature of the toxigenic *Aspergillus* moulds can result in the contamination of crops even when sophisticated pre- and post-harvest techniques are practised. The paucity of sophisticated operating procedures in Third World countries serves to exacerbate the aflatoxin problem. There is, therefore, an urgent need for procedures that enable aflatoxin-contaminated consignments of foods and feeds to be utilised whilst affording adequate protection to the consumer.

Aflatoxin-contaminated consignments of foods and feeds may be decontaminated either by removing the aflatoxin (segregation) or by converting the aflatoxin to a non-toxic form (degradation). Degradation may be achieved by physical, biological and chemical means.

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III. Segregation Procedures

Aflatoxin may be removed from contaminated commodities either by isolating the contaminated part of the consignment or by isolating the aflatoxin itself by, for example, solvent extraction.

1. The isolation of contaminated material

This technique may be practised in its simplest form by rejecting individual mouldy or discoloured kernels during the consumption of, for example, a packet of peanuts (groundnuts). The same principle is employed on a larger scale when complete consignments are subjected to visible inspection, as part of a quality control procedure. For example, a procedure has been described (Coker *et al.* 1985) for the rapid identification of *A. flavus* in samples collected from consignments of farmers' stock groundnuts, thus allowing the segregation of contaminated consignments.

Automatic electronic colour sorting, often in combination with manual sorting, is widely used to segregate kernels of abnormal colour or size during the processing of edible grade groundnuts. Colour sorting has also been used to segregate contaminated Fair Average Quality (FAQ) groundnuts which are normally used for oil extraction. FAQ kernels containing 1600 μg/kg aflatoxin have been sorted to afford a product with a contamination level of less than 10 μg/kg. However, the throughput was low and a rejection rate of the order of 20 per cent was required.

2. The isolation of aflatoxin

The isolation of aflatoxin from contaminated oilseed meals by solvent extraction has been widely investigated (Rayner *et al.* 1977). Early workers recognised that the conventional use of hexane to extract the oil from aflatoxin-contaminated cottonseeds and groundnuts served to concentrate the aflatoxin in the residual meal. It was also recognised that the use of an appropriate polar solvent, the presence of adequate water and operating temperatures close to the boiling point of the extraction solvents were important factors for the efficient extraction of the aflatoxin. Encouraging results were obtained using, for example, acetone:water (90:10), acetone:water:hexane (54:2:44), hexane:methanol (73:27), 2-propanol:water (80:20), and 95 per cent ethanol. A pilot-plant scale extractor has been used (Rayner *et al.* 1977) to reduce the aflatoxin content of a prepress solvent-extracted cottonseed meal from 300 to 2 μg/kg total aflatoxins, using the azeotrope of 2-propanol and water.

The combined extraction of the oil and aflatoxin from full-fat cottonseed and groundnut kernels has also been investigated. Mixtures of isopropanol and water (and of ethanol and water) were utilised in an experimental, continous countercurrent process (Karnofsky 1981). The use of methoxymethane:water (96:4) as an extraction solvent is an interesting development (Coker *et al.* 1984). Comminuted groundnut kernels and groundnut cake have been successfully treated at ambient temperature using an experimental semi-continuous process. Methoxymethane boils at -24.2°C and, consequently, was readily removed from the extracted oil. The simultaneous extraction of the oil and aflatoxin necessitated the decontamination of the oil.

The removal of aflatoxin by solvent extraction has not been exploited commercially despite the development of the procedures described above.

The isolation of aflatoxin from contaminated commodities, in liquid form, may also be performed by adsorption techniques. Groundnut oil has been decontaminated using activated kaolin (Miller *et al.* 1985). The removal of 79 per cent of the aflatoxin M_1 in raw milk contaminated at approximately 5 μg/kg has been reported using bentonite (0.4 g/20 ml) as the adsorption agent (Doyle *et al.* 1982).

IV. Physical Degradation

Attempts have been made to degrade aflatoxin by using various forms of radiant energy including heat, microwaves, gamma rays, X-rays and UV light.

It has been reported that the roasting of groundnuts reduces the level of aflatoxin contamination by approximately 30 per cent (Tiemstra 1977). The accurate estimation of the aflatoxin content of whole kernels before roasting remains a problem, however. The heat treatment of groundnut and cottonseed meals has also been investigated (Coker *et al.* 1984). An 80 per cent reduction in contamination was achieved by heating cottonseed meal (214 μg/kg) at 100°C for 2 hours. However when groundnut meal (111 μg/kg) was similarly treated a reduction in contamination of only 34 per cent occurred.

Groundnut and other oils are extensively used as cooking oils in Asian communities. Experiments with naturally contaminated groundnut oil and artificially contaminated corn oil showed that the degradation of aflatoxin occurs only at temperatures of 250°C and above. Normal frying temperatures are between 170 and 180°C (Peers & Linsell 1975).

Naturally contaminated groundnut kernels (56 μg/kg) were subjected to microwave irradiation. (Luter *et al.*1982). A 95 per cent reduction in contamination occurred within 5 minutes with an

operating temperature of approximately 150°C. The microwave treatment of contaminated groundnut cake has also been attempted; the reported reductions in contamination vary between 32 and 74 per cent (Grehaigne *et al.* 1983).

Gamma-irradiation is used for food preservation and its potential as an aflatoxin decontamination agent has been investigated. It was reported that the treatment of groundnut meal (2 mg/kg aflatoxin) destroyed all toxicity and mutagenicity towards the bacterium *Salmonella typhimurium*, strain TM 677 (Temcharoen & Thilly 1982).

Although X-rays have been used to destroy high levels of aflatoxin (2-3 mg/kg) in aqueous solution, the treatment of contaminated rice did not significantly reduce the aflatoxin content even after 12 months storage (Coker *et al.* 1984).

UV light has been used in attempts to degrade aflatoxin in milk, groundnut meal, groundnut protein, groundnut oil, coconut oil and copra. The UV-irradiation of milk (1 μg/kg aflatoxin M_1), circulating in a small water-cooled reaction vessel, completely degraded the aflatoxin after irradiating for 10 minutes in the presence of 1 per cent hydrogen peroxide (Yousef & Marth 1985).

The exposure of groundnut oil to bright sunlight, in India, degraded the aflatoxin within 15 minutes (Shantha & Murthy 1981). Similarly, the aflatoxin content of naturally contaminated coconut oil (100 μg/kg) was reduced to a level of less than 30 μg/kg when exposed, as a layer of 4 cm thickness, to bright sunlight (Cooker *et al.* 1984).

V. Biological Degradation

Over 1000 microorganisms have been screened for their ability to degrade aflatoxin B_1 *Flavobacterium aurantiacum* NRRL B-184 apparently degraded aflatoxin B_1 in aqueous solution as well as degrading the aflatoxins B_1 and G_1 in experimentally contaminated corn (28 mg/kg) and groundnuts (17 mg/kg). *F. aurantiacum* was also reported to degrade aflatoxin M_1 in naturally contaminated milk (10 mg/litre) (Marth & Doyle 1979). It was not clear whether the aflatoxin was simply adsorbed by the bacterial cells or whether actual degradation of the toxin occurred.

VI. Chemical Degradation

Numerous oxidising agents, aldehydes, acids and bases (inorganic and organic) have been investigated for the purpose of degrading aflatoxin (Grehaigne *et al.* 1983) and patented decontamination methods exist which utilise ammonia, calcium hydroxide, hydrogen peroxide, methylamine and a mixture of calcium hydroxide and methylamine. Formaldehyde and sodium bisulphite have also shown promise as decontamination agents. Although inorganic acids will convert aflatoxin B_1 to the much less toxic derivative, aflatoxin B_{2a}, the reaction proceeds slowly, under even fairly drastic conditions.

1. Oxidising agents

A combination of hydrogen peroxide (1 per cent) and riboflavin (0.5 mM) at 30°C inactivated 98 per cent of the aflatoxin M_1 in naturally contaminated milk (Doyle *et al.* 1982). Hydrogen peroxide, under alkaline conditions, has also been used to decontaminate groundnut meal and to produce aflatoxin-free protein isolate (Coker *et al.* 1984).

2. Aldehydes

Contaminated groundnut meal (570 μg/kg) has been treated under reflux conditions, with a formalin solution, to afford 99 per cent deactivation within 1 hour (Coker *et al.* 1984).

3. Inorganic and organic bases

Sodium bisulphite is commonly used a food preservative. When whole-kernel corn (2800 μg/kg aflatoxin) was treated with sodium bisulphite solution and incubated for 21 days, complete degradation of the aflatoxin occurred (Hagler *et al.* 1982).

The treatment of naturally contaminated corn (235 μg/kg; 20% moisture) with sodium hydroxide (2%), in aqueous solution, at ambient temperature for 24 hours reduced the level of contamination to below 20 μg/kg total aflatoxin (Doyle *et al.* 1982). Poultry feed, experimentally contaminated with 2000 μg/kg aflatoxin, was treated with the same concentration of sodium hydroxide before conditioning (at 80°C) and pelleting. Only 25 per cent decontamination occurred (Coker *et al.* 1984). The 95 per cent decontamination of groundnut meal (110 μg.kg; 30% moisture) and cottonseed meal (214 μg/kg; 22% moisture) was achieved with 2 per cent sodium hydroxide at 100°C within 2 hours (Grehaigne *et al.* 1983).

Erratic results were reported when contaminated cottonseed meal was treated with calcium hydroxide (Grehaigne *et al.* 1983). The decontamination of corn, using this reagent, was also unsuccessful (Alanis *et al.* 1981). However, the decontamination of copra (105 μg/kg aflatoxin) has been reported by adding the base as an aqueous slurry prior to pelleting (Coker *et al.* 1984).

Groundnut meal (110 μg/kg aflatoxin) has been successfully treated with methylamine (1.25%) at 100°C for 2 hours. A product with a slight 'fishy' aroma was reported (Grehaigne *et al.* 1983).

Groundnut meal (600 μg/kg) was also treated, on a pilot-scale, with calcium hydroxide (2%) and methylamine (0.5%) solution either in an unpressurised vessel (at 80-90°C) or in a heated screw-press extruder (at 100-110°C), to afford over 90 per cent decontamination. The dwell-time in the screw-press was approximately 5 minutes (Coker *et al.* 1984). Treatment with calcium hydroxide (2%) and formaldehyde (1%), in an unsealed reaction vessel, at an elevated temperature also degraded the aflatoxin in groundnut cake (Coker *et al.* 1984).

The application of ammonia to the decontamination of oilseed and cereal products has been very extensively investigated (Coker *et al.* 1985). Generally, the treatment involved the addition of water followed by ammonia gas, at an elevated temperature, to afford a pressure of 15-50 psi. For example, contaminated cottonseed meal (ca. 1000 kg; 545 μg/kg aflatoxin) was treated in a 18 x 3 ft diameter reaction vessel fitted with a double ribbon agitator, a centrifugal fan and a steam jacket. The moisture content was adjusted to 12.5 per cent, by the addition of water, and ammonia gas was then added for 30 minutes until a pressure of 45-50 psi was attained. The passage of steam through the steam jacket increased the temperature of the meal to about 71°C whereas the addition of ammonia elevated the temperature to 121°C. Finally, the ammonia was purged from the reactor using the centrifugal fan and monocalcium phosphate was added to the meal to adsorb any residual ammonia. No aflatoxin was detected in the final product (Coker *et al.* 1984); 95 per cent of the aflatoxin in low moisture-containing (7%), contaminated groundnut meal (1530 μg/kg) has been deactivated using ammonia gas in a steam-jacketed, stirred 50 kg capacity reaction vessel. A dwell-time of 15 minutes was reported, together with a pressure of approximately 44 psi and a temperature of 95°C (Coker *et al.* 1985). The aflatoxin content of 28 kg cottonseed meal (4000 μg/kg B_1; 14% moisture) has been reduced to less than 4 μg/kg within 30 minutes by treatment with 4 per cent ammonia gas at 40 psi and 100°C (Park *et al.* 1984).

Aqueous ammoniacal solutions have also been used to degrade aflatoxin in oilseed meals. Investigations have been performed under conditions of ambient temperature as well as during pelleting and extrusion operations. For example, 15 kg contaminated groundnut meal (450 μg/kg) was mixed with ammonia (6% NH_3) in aqueous solution, and stored for 2 weeks in plastic sacks to afford an apparent 98 per cent level of decontamination (Coker *et al.* 1985). The addition of an ammoniacal solution to contaminated groundnut meal prior to pelleting (Coker *et al.* 1984) or extrusion (Grehaigne *et al.* 1983) afforded 89 and 87 per cent reductions in aflatoxin content, respectively. Contaminated cottonseed meal (800 μg/kg) has also been successfully treated at ambient temperatures (ranging from 21 to 43°C) and atmospheric pressure (i.e. the so-called APAT method): 200g portions of the meal were mixed with ammonia (2%), in aqueous solution, and stored in sealed plastic bags for 15 days at a moisture content of 7.5 per cent. The treated meal contained less than 20 μg/kg aflatoxin (Coker *et al.* 1985). This decontamination method has been scaled-up for on-farm use; the cottonseed is treated in 10 x 10 x 100 ft. plastic 'Ag Bags'.

A process has been developed which allows the decontamination of 1000 bushels of whole corn at atmospheric pressure and ambient temperature (Coker *et al.* 1985). The treatment involved the adjustment of the moisture content (tempering), followed by the addition of ammonia gas and, finally, the drying of the corn to an acceptable moisture level. Each of these steps was performed in a separate storage bin, of about 2500 bushels capacity. The tempering bin was fitted with a stirring device and the ammoniation bin was carefully sealed in order to avoid ammonia leakage. The aflatoxin content of corn processed in this manner was reduced from 750 to 7 μg/kg over a period of approximately 15 days. Considerable safety precautions were required for the handling of both the untreated and treated corn.

VII. Processing Effects

The decontamination of aflatoxin-contaminated foods and feeds sometimes occurs during normal processing. The removal of aflatoxin during the refining of groundnut oil and the automatic electronic colour sorting of edible groundnut kernels serve to illustrate this point. Investigations into the fate of aflatoxin M_1 during the preparation of Parmesan cheese demonstrated that decontamination does not always occur during processing. For example, milk contaminated with 12 μg/litre aflatoxin M_1 afforded Parmesan cheese containing 64 μg/kg aflatoxin M_1.

VIII. The Commercial Usage of Decontamination Procedures

The segregation of contaminated foods and feeds, as part of a quality control procedure, is widely practised. For example, the quality control of edible groundnut kernels includes visual inspection together with sampling and analysis procedures. As mentioned previously, manual and/or automated sorting methods are also utilised during processing.

To date, solvent extraction has not been used, commercially, as a decontamination method. Similarly, physical degradation methods have not been exploited.

Ammonia and hydrogen peroxide have both been exploited for the chemical degradation of aflatoxin. The manufacture of protein isolate, in India, utilised hydrogen peroxide for the decontamination of groundnut protein. An output of 2 tons of isolate per day was reported. The ammoniation methods, developed in the USA, for the 'unofficial' on-farm decontamination of cottonseed (APAT method) and corn (sealed-bin method) have been described in a previous section.

The only techniques which have operated commercially are those developed by Lesieur Cotelle et Associes, in France, for the ammoniation of groundnut cake in Senegal, West Africa (Coker *et al.* 1985). The original process involved the sequential treatment of the cake with steam and ammonia gas in a stirred, 7 tonne capacity reaction vessel. Operating temperatures and pressures of 90°C and 30-40 psi, respectively, were used. The excess ammonia gas was removed by forced ventilation and recovered for future use. It appears that a new procedure using a mixture of ammonia and formaldehyde has now been introduced. Details of this procedure are not available but it is reported that lower operating pressures and ammonia concentrations are required.

IX. The Acceptability of Decontaminated Foods and Feeds

A decontamination procedure should provide a product which is non-toxic and which possesses acceptable nutritive, palatability, handling and processing characteristics. A large number of experiments have been performed to establish whether ammoniated cereals and oil-seed cakes are acceptable as animal feeds (Coker *et al.* 1984). The toxicity and nutritional quality of groundnut cake produced by the original Lesieur process has been extensively investigated (Coker *et al.* 1985). However, corresponding data for the new ammonia/formaldehyde method have not been published. No toxicological data have been obtained for the APAT method for the treatment of cottonseed. Ammoniated corn produce by the sealed-bin method has been subjected to toxicological evaluation, for submission to the USA Food and Drug Administration. However, FDA approval of this procedure is still awaited.

The chemical fate of aflatoxin in oilseeds and cereals, after ammoniation, has also been examined (Grove *et al.* 1984).

X. Procedures Developed at the Tropical Development & Research Institute

To date, the ammoniation of oilseeds and cereals has involved two approaches. The first approach is aimed at the rapid treatment of the feedstuff and is performed at pressures in excess of 15 psi and, typically, at 30-40 psi. The second approach involves ammoniation at atmospheric pressure and ambient temperature and requires a total treatment period of the order of 15 days.

The main objectives at TDRI were the development of a pilot-scale low pressure technique for the rapid ammoniation of groundnut cake, and the use of this technique in the preparation of diets for rat and dairy cattle feeding trials.

The optimum moisture and ammonia concentrations for the degradation of aflatoxin in groundnut cake were 15-20 and 3-4%, respectively. If the addition of ammonia gas was performed at ambient temperature, approximately 92% decontamination occurred after 7 days whereas a dwell-time of 22 days was required for 99% degradation of the aflatoxin. The addition of ammonia gas elevated the temperature of the cake to approximately 60°C. When the cake was held at this temperature, by using a Dewar flask, the dwell-time required for at least 95% decontamination was reduced, drastically, to 3-5 hours.

A further reduction in the required dwell-time was obtained by adding water in the form of steam and by operating under elevated pressures. The cake was not stirred during these experiments. The addition of 3% ammonia afforded a 94% reduction in contamination within 1 hour when maximum operating pressures and temperatures of 5 psi and 125°C, respectively, were used. If the reaction vessel was completely sealed, a maximum pressure of 22 psi was obtained. However, the achievement of 95% decontamination still required a dwell-time of 1 hour, under these conditions. The dwell-time was, however, reduced to 0.5 hours if 4% ammonia gas was added under completely sealed conditions. The corresponding maximum temperatures and pressures were 125°C and 27 psi, respectively.

An unstirred 53 kg reaction vessel was constructed and used for the successful decontamination of groundnut cake by the sequential addition of steam and ammonia gas. The maximum temperatures and pressures, during ammoniation, were 118°C and 7.5 psi, respectively, with a dwell-time of 1 hour. The toxicity of groundnut cake, decontaminated by this procedure, is currently under assessment (Coker *et al.* 1985).

A stirred reaction vessel of 0.5 tonne capacity is under construction and will be operated under a pressure not exceeding 10 psi. A continuous ammoniation device has also been constructed and commissioning trials are under way.

References

Alanis, G.; Elias, L.G.; Bressani, R. (1981) Effect of two alkaline treatments for the inactivation of aflatoxins in maize and their impact on biological assays. In *Annual Report of Institute of Nutrition of Central America and Panama*, 97-98.

Brackett, R.E.; Marth, E.H. (1982) Fate of aflatoxin M_1 in Parmesan and Mozzarella cheese. *Journal of Food Protection* **45**, 597-600.

Coker, R.D. (1979) Aflatoxin: past, present and future. *Tropical Science* **21**, 143-162.

Coker, R.D.; Jones, B.D.; Nagler, M.J.; Gilman, G.A.; Wallbridge, A.J.; Panigrahi, S. (1984) In *Mycotoxin Training Manual* Section A8. London; TDRI.

Coker, R.D.; Jewers, K.; Jones, B.D. (1985) The destruction of aflatoxin by ammonia; practical possibilities. *Tropical Science* **25**, 139-154.

Doyle, M.P.; Applebaum, R.S.; Brackett, R.E.; Marth, E.H. (1982) Physical, chemical and biological degradation of mycotoxins in foods and agricultural commodities. *Journal of Food Protection* **45**, 964-971.

Grehaigne, B.; Chouvel, M.; Pina, M; Graille, J.; Cheftel, J.C. (1983) Extrusion cooking of aflatoxin-containing peanut meal with and without addition of ammonium hydroxide. *Lebensmittelwissenschaft und Technologie* **16**, 317-322.

Grove, M.D.; Plattner, R.D; Peterson, R.E. (1984) Detection of aflatoxin D_1 in ammoniated corn by mass spectrometry-mass spectrometry. *Applied and Environmental Microbiology* **48**, 887-889.

Hagler, W.M.; Hutchins, J.E.; Hamilton, P.B. (1982) Destruction of aflatoxin in corn with sodium bisulfite. *Journal of Food Protection* **45**, 1287-1291.

Karnofsky, G (1981) Ethanol and isopropanol as solvents for full-fat cottonseed extraction. *Oil Mill Gazetteer* (April 1981), 34-36.

Luter, L; Wyslouzil, W.; Kashyap, S.C. (1982) The destruction of aflatoxins in peanuts by microwave roasting. *Journal, Institut Canadien de Science et Technologie Alimentaire* **15**, 236-238.

Marth, E.H.; Doyle, M.P. (1979) Update on moulds: degradation of aflatoxin. *Food Technology* (January), 81-87.

Miller, N.; de Villiers, J.B.M.; Pretorius, H.E. (1985) Detoxification of aflatoxin-containing crude groundnut oil. *Lebensmittelwissenschaft und Technologie* **18**, 201-202.

Park, D.L.; Lee, L.; Koltun, S.A. (1984) Distribution of ammonia-related reaction products in cottonseed meal. *Journal of American Oil Chemists' Society* **61**, 1071-1074.

Peers, F. G.; Linsell, C. A. (1975) Aflatoxin contamination and its heat stability in Indian cooking oils. *Tropical Science* **17**, 229-232.

Rayner, E.T.; Koltun, S.P.; Dollear, F.G. (1977) Solvent extraction of aflatoxins from contaminated agricultural products. *Journal of the American Oil Chemists' Society* **54**, 242A-244A.

Shanta, T.; Murthy, V.S. (1981) Use of sunlight to partially detoxify groundnut (peanut) cake flour and casein contaminated with aflatoxin B_1. *Journal of the Association of Official Analytical Chemists* **64**, 291-293.

Temcharoen, P.; Thilly, W.G. (1982) Removal of aflatoxin B_1 toxicity but not mutagenicity by 1 megarad gamma radiation of peanut meal. *Journal of Food Safety* **4**, 199-205.

Tiemstra, P.J. (1977) Aflatoxin control during food processing of peanuts. In *Mycotoxins in Human and Animal Health* (J. V. Rodricks; C. W. Hesseltine; M. A. Mehlman, eds), 121-137. Illinois; Pathotox Publishers.

Yousef, A.E.; Marth, E.H. (1985) Degradation of aflatoxin M_1 in milk by ultraviolet energy. *Journal of Food Protection* **48**, 697-698.

Spoilage and Mycotoxins of Cereals and other Stored Products.
Edited by B. Flannigan

Mycotoxins and the Fermentation Industries

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I. Summary

Normally, only barley with low levels of mycotoxins is malted in UK, but imported adjuncts may contain higher levels. In developing countries, grain which is more heavily contaminated is often used for production of alcoholic beverages. Some mycotoxins may be destroyed during malting, but others may be produced by toxigenic species which are able to grow during malting. Although there is no firm evidence, mycotoxins in alcoholic beverages are considered by some to be contributory causes of various types of cancer and other human disorders. Spent grains, in which mycotoxins accumulate, and other residues used as animal feeds may cause serious mycotoxicoses in stock. Trichothecenes are phytotoxic and experiments show that they can affect malting of barley. Trichothecenes and some other mycotoxins retard yeast growth and fermentation.

II. Introduction

The requirements of the UK brewing and distilling industries are such that normally only high-quality barley is used. As far as can be gathered, mycotoxin levels in home-grown barley for malting are low. For example, although Gilbert *et al.* (1983) found that deoxynivalenol (DON) was fairly common, in none of the samples did the concentration exceed 0.1 mg/kg, the level which they considered to be of significance. In imported flaked maize and maize grits, the average was 0.06 mg/kg, and only two of the 10 samples examined contained >0.1 mg DON/kg. Levels in whole maize imported for food processing were somewhat higher than in the maize flake and grits, which, until a levy was recently placed on maize imported from outside the EEC, were frequently used by the UK fermentation industries as adjuncts providing fermentable carbohydrate. Although some imported material might therefore contain higher concentrations of mycotoxins than in home-grown grain, these levels are much less than may be present in grain used for fermentations in some areas of the world.

III. Mycotoxins in brewing materials and beer

In areas of Africa, better quality grain is retained for food and grain that is noticeably colonized by moulds is used either for animal feed or for the brewing of local beer, sometimes being preferred for brewing because of supposedly better flavour (Marasas *et al.* 1979). In Zambia, Lovelace & Nyathi

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(1977) examined commercial and home-brewed opaque maize beer made from grain which was extensively invaded by *Fusarium graminearum*. Both the maize and the malt prepared from it were heavily contaminated with zearalenone. The mean concentration of zearalenone in the beer was 0.2 mg/litre, and in one sample of home-brewed beer 4.6 mg/litre was detected. The mean toxin level in maize used for production of commercial beer was 0.33 mg/kg, and in the beer itself 0.34 mg/litre. Although the toxin was present in the large amounts of solids such beer traditionally contains, it was found predominantly in the liquid fraction. In Swaziland, Martin & Keen (1978) found that of 55 samples of local alcoholic drinks, sour porridges and beers produced by fermentation of various combinations of maize or sorghum meal, flour and sorghum malt, six contained zearalenone, at a level equivalent to 8-53 mg/kg. In Lesotho, 17 of 140 samples of joala beer, prepared from malted maize or sorghum, flour and hops, contained zearalenone (0.3-2.0 mg/kg), but no aflatoxin, ochratoxin or sterigmatocystin was detected.

Lovelace & Nyathi (1977) found aflatoxin in only one lot of malted maize, but there was none in the beer made from this, and in a population-based study in Kenya Peers & Linsell (1973) detected aflatoxin in only 16 of the samples of beer collected from 304 centres (1.0-2.5 μg/litre). The latter authors expressed surprise at the low incidence of aflatoxin in the local beer, considering that rejected grain was used for brewing, and suggested that it was possibly degraded during fermentation. Although Mann & Rehm (1977) reported that *Saccharomyces cerevisiae* does not degrade aflatoxin, it is likely that other yeasts which do, e.g. *Candida* spp., will be present during such fermentations. In contrast to these investigations, a study of indigenous alcoholic beverages in Nigeria (Alozie *et al.* 1980) indicated that aflatoxin was frequently present in palm wines and sorghum- or millet-based beers. Samples of the wines contained 83-86 μg/litre, and the beers 92-256 μg/litre. These beverages may be stored unsterile for up to eight days before consumption and all were found to support growth and toxin production by *Aspergillus flavus*, >1 mg/litre being detected in burukutu beer stored for such a period after inoculation with *A. flavus*.

When the fate of mycotoxins in the closely controlled malting and brewing processes of Western countries is considered, it is found that there is some disparity between different investigations. When Krogh *et al.* (1974) malted barley lots which were moderately contaminated with ochratoxin A (0.42-0.83 mg/kg), they failed to detect any toxin in the malt. With highly contaminated barley (2.0-27.5 mg/kg), which would have been rejected for commercial malting because of its weathered appearance alone, it was necessary to prepare a wort for fermentation by using bacterial enzymes, since the grain showed very low germination and could not be malted. The wort contained only 11-19% of the original toxin and only 2-7% was recovered from the beer brewed from this, whilst 6-10% was found in the spent grains. In contrast, when citrinin was added to a mash prepared from uncontaminated barley malt, neither the wort collected by filtration of the mash nor the spent grains contained any of that toxin. However, when a mash was spiked with ochratoxin 16% was found in the wort, 4% in the beer and 10% in the spent grains.

Chu *et al.* (1975) recovered proportionally much more toxin in their micro-brewing experiments. They found that ochratoxin A and alfatoxin B_1 were heat stable, and in the boiled wort recovered 48-54% of the ochratoxin and 40-49% of the aflatoxin added at mashing (1-10 mg/kg). The corresponding values for the beer were 14-28% and 18-27%, respectively. In later experiments (Nip *et al.* 1975), 28-39% of the labelled ochratoxin was found in the spent grains, 8-20% in the yeast and 14-18% in the beer. It was concluded that 38% of the toxin added at the rate of 1 mg/kg was degraded, and 12% of that at the 10 mg/kg level.

From experiments in which the fusarial toxins T-2 and zearalenone were added to barley before malting, Flannigan *et al.* (1985) concluded that most toxin was lost or degraded during the malting process. Only traces of the added T-2 and 9-13% of the zearalenone were detected in kilned malt. Approximately 39-54% of the former toxin and 8-18% of the latter were detected in the water in which the barley had been steeped. Nevertheless, in a survey of European light lagers (Payen *et al.* 1983), a few samples contained T-2 (3/49) and zearalenone (1/49), and DAS (2/49), DON (1/49) and ochratoxin (4/37) were also detected.

It is not for their mycotoxins that fusaria are best known in the brewing industry. Heavy contamination of barley by some *Fusarium* spp. causes pronounced changes in the characteristics of the malt produced, and beer prepared using this malt is often noted for 'gushing'. Since gushing is a particular problem in Scandinavia, Haikara (1983) investigated the problem by artificially contaminating barley in the field with fusaria. She found that many changes in the quality of the malt could be regarded as improvements, but there were marked differences between the effects of different *Fusarium* isolates. Although a causal relationship was not demonstrated, strains of *F. culmorum* which had the most severe effects on germination of the barley and caused the greatest gushing were also abundant producers of zearalenone. They contrasted in this last respect with *F. avenaceum* strains. Many of the barley lots heavily colonized by these fusaria would have been rejected for commercial malting on account of their low germinative capacity. However, when the barley was malted experimentally, those lots colonized by *F. culmorum* yielded malt containing 10 and 30 mg zearalenone/kg, compared with 0.2 and 9 mg/kg in the original barley. Assays showed that three other fusarial toxins, viz. T-2, HT-2 and neosolaniol, were not present in either barley or malt. It must be presumed that a malting procedure in which heavily colonized grain is steeped at 12°C for

28-30 h and allowed to germinate at 15° for seven days provides favourable conditions for growth and zearalenone production by fusaria. However, conditions appear to be less favourable for production of the nephrotoxins ochratoxin A and citrinin (Gjertsen *et al.* 1973). Little or no toxin was found in malt (or beer) after toxigenic strains of *Aspergillus ochraceus, A. niveus* and *Penicillium viridicatum* were added at steeping.

IV. Health risks

Although mycotoxins are present in at least some alcoholic beverages, the health risk to humans drinking these is unknown. Although Payen *et al.* (1983) found trichothecenes, zearalenone and ochratoxin in European lagers, they concluded that the low levels would present no hazard, unless perhaps to heavy drinkers. Cook & Collis (1972) found that there was no statistical or epidemiological evidence to suggest a link between aflatoxin in maize beer and the incidence of oesophageal cancer in East Africa. However, Martin & Keen (1978) suggested that the methods of making beer in Lesotho might have serious implications for health. They noted that a survey indicated that there was a greater intake of indigenous fermented products and European alcoholic drinks among patients with cervical cancer than among healthy subjects. A possible link between the incidence of oesophageal cancer in Transkei and DON and zearalenone in maize used for beer has also been suggested (Marasas *et al.* 1979). In this context, it is worth noting that, from the toxin levels and the pattern of consumption of opaque maize beer in different districts, Lovelace & Nyathi (1977) concluded that the mean daily intake of zearalenone among consumers of this type of beer in Zambia would be 0.17-0.45 mg.

The possibility that zearalenone in alcoholic beverages is a cause of tumour development in the reproductive and certain other target organs, and that zearalenone and trichothecenes were involved in 'cobalt beer drinker's cardiomyopathy', was discussed by Schoental (1980, 1984). She suggested that, whilst foetal abnormalities and various other disorders are attributed to alcohol *per se*, mycotoxins and nitroso compounds contaminating alcoholic beverages might be involved. Large doses of oestrogens are known to cause cancer of the reproductive organs, and zearalenone attaches itself to the same cellular receptors as other oestrogenic agents. It is carcinogenic in experimental mice and teratogenic in rats. Schoental (1984) found that zearalenone, alone or in combination with T-2 toxin, induced tumour development in the pituitary, endometrium, prostrate gland and testes of experimental rats. She also suggested that the absence of zearalenone in most of the beers examined by Payen *et al.* (1983) could have been due to reduction to zearalenols during fermentation. Although alpha-zearalenol is 3x more active than zearalenone, it is less frequently looked for in surveys. Schoental (1984) suggested that the presence of either compound in the body might raise the overall amount of oestrogenic agents in the body to a level which could induce tumour development. Canavese *et al.* (1981) raised the question of possible involvement of mycotoxins in renal diseases of previously unknown aetiology.

What appeared to be a natural intoxication due to consumption of commercial beer was reported in US by Cole *et al.* (1983). Some 4 h after consuming the beer, the individual became acutely ill with frontal headache, double vision, fever, weakness, nausea, vomiting and bloody diarrhoea; after 12 h, he could not write legibly owing to tremor; after 30 h, the condition had resolved itself. *Penicillium crustosum* was isolated from a pellicle-like mycelium within the otherwise normal can. When grown on a liquid medium containing commercial beer, the isolate produced the alkaloids roquefortine, roquefortine A, roquefortine B and festuclavine, although in smaller amounts than on solid medium. However, it was not possible to determine whether these compounds were involved in the intoxication, since none of the original beer remained for analysis.

V. Mycotoxins in by-products of malting and brewing

The use of by-products of the fermentation industries for animal feed may lead to mycotoxicoses in stock. Perhaps the most serious mycotoxicosis caused by such a by-product was an outbreak of fatal tremorgenic disease among cattle which had been fed on residues from production of sorghum beer in South Africa (Kellerman *et al.* 1976). The residues were heavily contaminated with *Aspergillus clavatus*, which produces the tremorgenic toxins tryptoquivaline and tryptoquivalone and is not uncommon in sorghum malt. It is not clear, however, whether growth of the mould during malting or when the residues were spread out on a floor to dry was responsible for the toxicosis. In reviewing the production of industrial ethanol from distressed or contaminated grain, Hesseltine (1984) stated that mycotoxins can be present in spent grains at concentrations which make them unsuitable for animal feed unless they are detoxified in some manner. His colleagues at the Northern Regional Research Centre of US Department of Agriculture (Lillehoj 1978, Lillehoj *et al.* 1979) found that, although the ethanol produced after enzymic digestion of the starch in aflatoxin-contaminated maize contained no toxin, toxin levels (w/w) in the residual solids were higher than in the original grain. This was the case in zearalenone-contaminated maize also. Little of the zearalenone originally present

(33.5 mg/kg) was degraded; the level detected in the distiller's solubles was 14-20 mg/kg, and in the solids 50-62 mg/kg (Bennett *et al.* 1981). As was mentioned previously, ochratoxin A has been recovered from spent grains after mashing of barley malt for beer production (Krogh *et al.* 1974), and, when Mannio & Enari (1973) malted barley heavily contaminated with fusaria, mice fed on spent grain developed typical symptoms of fusariotoxicosis. The beer produced from this malt contained no zearalenone or T-2 and was not toxic, but Mannio & Enari (1973) advised that spent malt grains originating from such barley should not be used as feeding stuff.

The rootlets and coleoptiles, or 'culms', screened off after malt is kilned are also used for animal feed and may be a cause of mycotoxicoses. There have been several reports of intoxication among cattle fed on culms contaminated by *Aspergillus clavatus*; these have been reviewed by Flannigan (1986). *A. clavatus* is well adapted to growth during malting, and is the causal agent of the allergic respiratory disease known as malt worker's lung. Some sufferers from this condition also exhibit symptoms suggestive of pulmonary mycotoxicosis. In addition to the tremorgens tryptoquivaline and tryptoquivalone mentioned earlier, *A. clavatus* also produces nortryptoquivaline, deoxytryptoquivaline, deoxynortryptoquivaline, deoxynortryptoquivalone, patulin, ascladiol and cytochalasins E and K, which have also been found in other materials in which it has grown. Apta *et al.* (1984) reported that 25% of the inoculum of this species on green malt survived kilning, and 5-7% was present in the culms. As both culms and finished malt are hygroscopic, the presence of this inoculum could initiate growth and possibly toxin production under poor storage conditions. Flannigan *et al.* (1986a) demonstrated that *A. clavatus* could grow on malt stored at high relative humidity. It is also worth noting that *A. flavus* could also grow on malt, and produce aflatoxins, under such conditions.

VI. Effects of mycotoxins on malting performance

The phytotoxicity of trichothecenes is well known (Ueno, 1984), and it is therefore not surprising that experimental studies have shown that malting of barley is affected by these toxins. By adding T-2 before malting, Nummi *et al.* (1975) were able to demonstrate that there was a concentration-dependent inhibition of rootlet and coleoptile elongation in germinating acid-dehusked barley. At the highest of the unspecified concentrations, elongation was entirely suppressed. However, in intact barley kernels the effects of T-2 were less severe, indicating that the husk had some mitigating effect. Ylimaki *et al.* (1979) found that *Fusarium*-inoculated barley with a T-2 level of 0.02 mg/kg had a germinative capacity, or percentage of viable embryos, of only 74%. When Flannigan *et al.* (1985) applied T-2 toxin to barley at the much higher rate of 10 mg/kg before steeping, however, there was no effect on either the germinative capacity or the germinative energy (percentage germination). Nevertheless, inhibitory effects on rootlet and coleoptile elongation similar to those reported by Nummi *et al.* (1975) were again observed. It is possible that the severe effects on the viability of embryos reported by Ylimaki *et al.* (1979) were due not only to T-2, since other metabolites produced by the fusaria on the grain may have caused germ damage. In other experiments employing the same methods as Flannigan *et al.* (1985), DAS was found, like T-2, to inhibit coleoptile and rootlet growth, but have no effect on germinative energy (Ferguson *et al.* 1984).

It could be expected that the *de novo* synthesis of alpha-amylase in germinating barley would be affected by trichothecenes, as they are noted for inhibiting protein synthesis (Ueno, 1984). Nummi *et al.* (1975) reported that there was a concentration-dependent retardation of development of this enzyme. In experimental malt produced from barley at the highest (unspecified) concentration of T-2 the alpha-amylase activity was only 10% of that in controls. Flannigan *et al.* (1985) also presented results indicating that T-2 inhibited synthesis of the enzyme, but found that addition of 10 mg T-2/kg barley before malting reduced activity in the kilned malt by only 23%. Ferguson *et al.* (1984) noted a similar effect with DAS. Flannigan *et al.* (1985) found that the alpha-amino nitrogen content of wort prepared from this malt was reduced by 9%. Again the reduction may have been due to repression of enzyme synthesis, since some of the proteolytic enzymes which by their action contribute to the alpha-amino nitrogen level of wort, are synthesised during malting. The reduction in the level of enzyme activity caused by the toxin appeared to have little effect on the modification of the starchy endosperm during malting, however, since the S.G. of the wort prepared by mashing the malt in water did not differ from that of the control.

Clearly, the concentrations of toxins used in these studies are higher than is likely to be encountered in malting barley, but they indicate the potential of the trichothecenes to affect malting. It is also to be remembered that in nature more than one toxin is likely to be present. For example, in addition to trichothecenes, zearalenone may be present. This toxin, which appears to affect the plasma membrane of the cell in plants (Vianello & Macri 1978), was considered to be the toxic component in extracts of grain infected with *Fusarium graminearum* which inhibited growth of maize embryos (Brodnik 1975). It is possible that, together with other physiologically active metabolites produced by the fungi on kernels, low concentrations of mycotoxins acting synergistically could affect malting performance.

VII. Effects of mycotoxins on yeast growth and fermentation

Fermentative yeasts are also affected by mycotoxins. Both Siriwardana (1980) and Lafont *et al.* (1981) reported that aflatoxin, patulin and T-2 toxin depressed fermentation velocity, and the latter authors found that DAS acted similarly. Considerably earlier, Mayer & Legator (1969) found that patulin, a carcinogen produced by a number of aspergilli and penicillia associated with cereals, caused extrachromosomal mutation in *Saccharomyces cerevisiae*, producing respiratory-deficient petite mutants. However, Sumbu *et al.* (1983) observed that the effects of patulin on cultures were transient, and the toxin was subsequently degraded. They suggested that patulin was degraded by an inducible enzyme system, and indicated that induced cells were resistant to very high concentrations of the toxin. Stinson *et al.* (1979) had earlier reported that the amount of patulin in apple juice was reduced to $<1\%$ of the original levels during fermentation.

Schappert & Khachatourians (1983, 1984) and Sukroongreung *et al.* (1984) have demonstrated that T-2 inhibits growth of *S. cerevisiae, S. carlsbergensis* and *S. diastaticus*, which are all used in alcohol fermentations. It has also been reported that logarithmic growth of *S. cerevisiae* is inhibited to varying extents by DAS (Ferguson *et al.* 1984), T-2 and zearalenone (Flannigan *et al.* 1985). Both T-2 and DAS affect viability, but if cultures of *S. cerevisiae* are allowed to incubate for 20 h or more, complete recovery of growth occurs at lower concentrations of toxin and there is partial recovery at higher concentrations (Flannigan *et al.* 1986b).

Although Lafont *et al.* (1981) found that T-2 and DAS had a much greater effect on fermentation velocity than aflatoxin or patulin, they did not draw attention to that the fact that their results indicated that there was some recovery from the effects of the trichothecenes towards the end of their 4-h test. In longer-term fermentations (Ferguson *et al.* 1984; Flannigan *et al.* 1986b), with DAS and T-2 there is a concentration-dependent lag in attenuation of glucose and production of ethanol, but thereafter the rate of both recovers to that of controls and there is finally little difference in amounts of ethanol produced. Flannigan *et al.* (1986b) have concluded that these toxins have the potential to retard commercial fermentations, whether these are for beer, whisky or fuel alcohol production. It is possible that such mycotoxins could be a contributory factor in 'sticking' fermentations, but additional work mentioned by these authors showed that increasing the pitching rate (inoculum density), or even adding dead cells along with the viable yeast inoculum, reduced the lag in fermentation. Starting with trichothecene-resistant membrane mutants (Schappert & Khachatourians 1984), an alternative strategy for fermentation of heavily contaminated grain for fuel ethanol would be to develop fast-fermenting trichothecene-resistant strains.

References

Alozie, T.C.; Rotimi, C.N.; Oyibo, B.B. (1980) Production of aflatoxin by *Aspergillus flavus* (UBMI) in some Nigerian indigenous beverages and foodstuffs. *Mycopathologia* **70**, 125-128.

Apta, R.; Flannigan, B.; McFarlane, G.B. (1984) Studies on *Aspergillus clavatus* and the malting of barley. *Mycological Society of America Newsletter* **35** (1), 18.

Bennett, G.A.; Lagoda, A.A.; Shotwell, O.L.; Hesseltine, C.W. (1981) Utilization of zearalenone-contaminated corn for ethanol production. *Journal of the American Oil Chemists Society* **58**, 974-976.

Brodnik, T. (1975) Influence of toxic products of *Fusarium graminearum* and *Fusarium moniliforme* on maize seed germination and embryo growth. *Seed Science and Technology* **3**, 691-696.

Canavese, C.; Stratta, P.; Ceruti, A.; Vercellone, A. (1981) Beer drinker's kidney: a mycotoxicosis? *Lancet* **8250**, 810-811.

Chu, F.S.; Chang, C.C.; Ashoor, S.H.; Prentice, N. (1975) Stability of aflatoxin B_1 and ochratoxin A in brewing. *Applied Microbiology* **29**, 313-316.

Cole, R.J.; Dorner, J.W.; Cox, R.H.; Raymond, L.W. (1983) Two classes of alkaloid mycotoxins produced by *Penicillium crustosum* Thom isolated from contaminated beer. *Journal of Agricultural and Food Chemistry* **31**, 655-657.

Cook, P.; Collis, C.H. (1972) Cancer of the oesophagus and alcoholic drinks in East Africa. *Lancet* **7758**, 1014.

Ferguson, P.E.; Flannigan, B.; Schapira, S.F.D. (1984) Effects of diacetoxyscirpenol on malting and yeast growth and fermentation. *SIM News* **34** (4), 45-46.

Flannigan, B. (1986) *Aspergillus clavatus* - an allergenic, toxigenic deteriogen of cereals and cereal products *International Biodeterioration* **22**, 79-89.

Flannigan, B.; Morton, J.G.; Naylor, R.J. (1985) *Fusarium* mycotoxins and the malting of barley. In *Trichothecenes and other mycotoxins* (J. Lacey, ed.), 171-184. Chicester; John Wiley.

Flannigan, B.; Healy, R.E.; Apta, R. (1986a) Biodeterioration of dried barley malt. In *Biodeterioration 6* (S. Barry; D. Houghton; G.C. Llewellyn; C. O'Rear, eds.), 300-305. Farnham Royal,; Commonwealth Agricultural Bureaux.

Flannigan, B.; Naylor, R.J.; Prescott, G.M.; Schapira, S.F.D. (1986b) Effects of trichothecene mycotoxins on yeast growth and fermentation. In *Biodeterioration 6* (S. Barry, D. Houghton; G.C. Llewellyn; C. O'Rear, eds.), 238-243. Farnham Royal; Commonwealth Agricultural Bureaux.

Gilbert, J.; Shepherd, M.J.; Startin, J.R. (1983) A survey of the occurrence of the trichothecene mycotoxin deoxynivalenol (vomitoxin) in UK grown barley and in imported maize by combined gas chromatography-mass spectroscopy. *Journal of the Science of Food and Agriculture* **34**, 86-92.

Gjertsen. P.; Myken, F.; Krogh, P.; Hald, B. (1973) Malting and brewing experiments with ochratoxin and citrinin. *Proceedings of the European Brewery Convention Congress* **14**, 373-380.

Haikara, A. (1983) Malt and beer from barley artificially contaminated with *Fusarium* in the field. *Proceedings of the European Brewery Convention Congress* **19**, 401-408.

Hesseltine, C.W. (1984) Mycotoxins and alcohol production: a review. In *Toxigenic fungi - their toxins and health hazard* (H. Kurata; Y. Ueno, eds.), 153-161. Tokyo; Kodansha/Elsevier.

Kellerman, T.S.; Pienaar, J.G.; van der Westhuizen, G.C.A.; Anderson, L.A.P.; Naude, T.W. (1976) A highly fatal tremorgenic mycotoxicosis of cattle caused by *Aspergillus clavatus*. *Onderstepoort Journal of Veterinary Research* **43**, 147-154.

Lafont, J.; Romand, A.; Lafont, P. (1981) Effets de mycotoxines sur l'activite fermentaire de *Saccharomyces cerevisiae*. *Mycopathologia* **74**, 119-124.

Lillehoj, E.B. (1978) Use of mycotoxin contaminated grain in the ethanol fermentation process. *Proceedings of the Distillers Feed Research Council* **33**, 23-29.

Lillehoj, E.B.; Lagoda, A.A.; Maisch, W.F. (1979) The fate of aflatoxin in naturally contaminated corn during ethanol fermentation. *Canadian Journal for Microbiology* **25**, 911-914.

Lovelace, C.E.A.; Nyathi, C.B. (1977) Estimation of fungal toxins, zearalenone and aflatoxin,contaminating opaque maize beer in Zambia. *Journal of the Science of Food and Agriculture* **28**, 288-292.

Mann, R.; Rehm, H.J. (1977) Degradation of aflatoxin B_1 by various microorganisms. *Zeitschrift für Lebensmitteluntersuchung und -forschung* **163**, 39-43.

Mannio, M.; Enari, T.-M. (1973) Über die Wirkung von *Fusarium*-Schimmelpilzen der Gerste bei der Bierherstellung. *Brauwissenschaft* **26**, 134-137.

Marasas, W.F.O.; van Rensburg, S.J.; Mirocha, C.J. (1979) Incidence of *Fusarium* species and the mycotoxins, deoxynivalenol and zearalenone, in corn produced in esophageal cancer areas in Transkei. *Journal of Agricultural and Food Chemistry* **27**, 1108-1112.

Martin, P.M.D.; Keen, P. (1978) The occurrence of zearalenone in raw and fermented products from Swaziland and Lesotho. *Sabouraudia* **16**, 15-22.

Mayer, V.W.; Legator, M.S. (1969) Production of petite mutants of *Saccharomyces cerevisiae* by patulin. *Journal of Agricultural and Food Chemistry* **17**, 454-456.

Nip, W.K.; Chang, F.C.; Chu, F.S.; Prentice, N. (1975) Fate of ochratoxin in brewing. *Applied Microbiology* **30**, 1048-1049.

Nummi, M.; Niku-Paavola; Enari, T.-M. (1975) Der Einfluss eines *Fusarium*-toxin auf die Gersten-Vermalzung. *Brauwissenschaft* **28**, 130-133.

Payen, J.; Girard, T.; Gaillardin, M.; Lafont, P. (1983) Sur la presence de mycotoxines dans des bieres. *Microbiologie Aliments Nutrition* **1**, 143-146.

Peers, F.G.; Linsell, C.A. (1973) Dietary aflatoxins and liver cancer - a population based study in Kenya. *British Journal of Cancer* **27**, 473-484.

Schappert, K.T.; Khachatourians, G.G. (1983) Effects of fusariotoxin T-2 on *Saccharomyces cerevisiae* and *Saccharomyces carlsbergensis*. *Applied and Environmental Microbiology* **45**, 862-867.

Schappert, K.T.; Khachatourians, G.G. (1984) Influence of the membrane on T-2 toxin toxicity in *Saccharomyces*. *Applied and Environmental Microbiology* **47**, 681-684.

Schoental, R. (1980) Relationship of *Fusarium* mycotoxins to disorders and tumours associated with alcoholic drinks. *Nutrition and Cancer* **2**, 88-92.

Schoental, R. (1984) Zearalenone, its biological and pathological, including carcinogenic, effects in rodents: implications for humans. In *Proceedings of the Fifth Meeting on Mycotoxins in Animal and Human Health, Edinburgh, August 1984* (M.O. Moss, M. Frank, eds.), 62-67. Guildford; University of Surrey.

Siriwardana, M.G. (1980) Les effets des mycotoxines sur *Saccharomyces cerevisiae*. PhD thesis, University of Caen.

Stinson, E.E.; Osman, S.F.; Huhtanen, C.N.; Bilis, D.D. (1978) Disappearance of patulin during alcoholic fermentation of apple juice. *Applied and Environmental Microbiology* **36**, 620-622.

Sukroongreung, S.; Schappert, K.T.; Khachatourians, G.G. (1984) Survey of sensitivity of twelve yeast genera toward T-2 toxin. *Applied and Environmental Microbiology* **48**, 416-419.

Sumbu, Z.L.; Thonart, P.; Bechet, J. (1983) Action of patulin on yeast. *Applied and Environmental Microbiology* **45**, 110-115.

Ueno, Y. (1983) *Trichothecenes: chemical, biological and toxicological aspects*. Amsterdam; Elsevier.

Vianello, A.; Macri, F. (1978) Inhibition of plant cell membrane transport phenomena induced by zearalenone (F-2). *Planta* **143**, 51-57.

Ylimaki, A.; Koponen, H.; Hintikka, E.-L.; Nummi, M.; Niku-Paavola, M.-L.; Ilus, T.; Enari, T.-M. (1979) *Mycoflora and occurrence of Fusarium toxins in Finnish grain*. Materials and Processing Technology, Publication 21. Espoo; Technical Research Centre of Finland.

Spoilage and Mycotoxins of Cereals and other Stored Products.
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TECHNIQUES FOR INVESTIGATION OF STORED PRODUCTS

New Developments in the Accurate Identification of Aspergilli in Stored Products

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I. Summary

The aspergilli associated with stored cereal spoilage and mycotoxin production are contained within eleven so-called species groups made up of some 130 species. Both group and species diagnosis are based on subjective decisions to determine or differentiate similarities in colony colour and on morphological features as identified by the inadequate resolution of the light microscope. Thus traditional methods are insufficient for the accurate determination of species which is vital to the success of mycotoxin research and diagnosis. This paper illustrates how the scanning electron microscope, involving a comprehensive study of conidial genotype and phenotypic variability, has resolved the problem. Two techniques are described which have been developed to permit accurate and rapid identification to specific level of aspergilli present *in situ* on the product, without recourse to plate culturing. Examples are provided of how, using traditional methods, misidentifications of important species can easily occur, and how the use of micromorphological differences in conidial ornamentation, as seen by the scanning electron microscope, can be used to solve them. Scanning electron micrographs illustrating distinctive conidial features of those mycotoxin producing species are provided and where necessary compared with those close relatives with which they are most often confused.

II. Introduction

The last major revision (Raper & Fennell 1965) divided the genus *Aspergillus* into 18 species groups comprising 151 species. Since then other minor revisers have erected a further 11 genera to accommodate these same taxa (Malloch & Cain 1972, Subramanian 1972). The entire known flora of food products is represented by about 130 species contained in 11 of these species groups, or 8 genera, depending on the classification to which one ascribes.

The presence of *Aspergillus* species in durable foodstuffs, especially cereals and cereal products, undoubtedly is a prime cause of their deterioration and spoilage during storage, a fact well supported by other contributors to these proceedings. The theme and contents of the proceedings also highlight the need to make accurate and speedy species determination in order to monitor and evaluate toxic and non-toxic fungi present in foods for human and animal consumption. Species identification is equally important when defining correct drying and storage parameters.

III. Traditional Methods of Identification

Despite three major taxonomic revisions in the past sixty years (Thom & Church 1926, Thom & Raper 1945, Raper & Fennell 1965) species identification is largely unreliable; its implementation frequently resembling an art form rather than scientific endeavour. Often, and this is an important point to which I will return, the identifier is unaware that he is in error. The reason lies in the nature of the *Aspergillus* organism itself in which those anatomical features employed as

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taxonomic characters are so microscopic that their proper resolution is beyond the limits of light microscopy. Thus, identifiers tend to rely heavily upon differences in plate colony characteristics visual to the human eye, particularly colony colour, together with the form of individual conidial heads. These characters are used for both group and species determinations with the identification often stopping at the group level.

When the contrast between colony colours is extreme, e.g. black or white, boundaries between taxa are said to be discrete. But, in general, taxon differentiation depends upon subtle differences between shades of the same colour, which introduces a strong subjective element to one's choice. The problem of subjective choice is further complicated since colour often varies with respect to the growth medium. It may even vary unaccountably under constant growth conditions. Additionally, mutant strains of the same species are a particular problem in that a green genotype can produce mutants which may be white, brown or red in colour.

The compound microscope is used to distinguish differences in the morphology of the conidial head, particularly the uniseriate and biseriate arrangement of the conidiogenous cells on the vesicle, and the shape of the vesicle itself. However, using these methods, in respect of all these features the phenotype is again highly variable.

In summary, the characters to which I have referred, traditionally used to identify the aspergilli do not exhibit sufficient discontinuity in their variation to provide completely reliable marker characters at specific or group level.

However, one other feature remains to be considered, namely the spore, termed the ascospore when referring to the sexual or teleomorph stage, and conidium for the asexual or anamorph stage. Because ascospores are sexual elements and because they are the slightly larger of the two spore forms, in species identification heavy reliance has been placed upon their ornamental differences. However, these are only just visible with the light microscope and the use of this character is further limited because few species possess a sexual stage. On the other hand, the asexual form is common to all and conidia are produced in abundance, but their size is such that using light microscopy they can at best be classified as smooth or rough (Fig. 1a and c).

IV. Scanning Electron Microscopy

The advent of the scanning electron microscope (SEM) with such a profound improvement in resolution, appeared to me to herald a new era in *Aspergillus* taxonomy introducing key diagnostic characters based on differences in ornamentation of the ubiquitous conidium (Fig. 1b and d).

The validity of this new criterion has been tested in an extensive study of the intraspecific and interspecific variation exhibited by some 105 species associated with stored foods. This has involved comparing variance in species subjected to physiological and environmental stress as well as considering geographic, ecological and mutational variance. As a consequence it had been demonstrated that the genetic integrity of the ornamentation pattern is maintained under all conditions cited above (Kozakiewicz 1983).

The range of conidial ornamentation has been classified into nine categories varying from echinulate to smooth (Kozakiewicz 1983). Within each category discontinuity in variability is such that distinct morphoforms are recognisable. In the majority of cases these are unique to one species, so that the application of this knowledge has a direct practical value for species identification.

Earlier, I remarked upon problems associated with identifications based on colony colours. This is particularly apt when the colour is distinctive and is associated with a commonly occurring species. For example, I have encountered problems with identified samples sent to me for verification, in which black isolates have been misidentified, and where white colonies without further consideration have automatically been assigned to *Aspergillus candidus*. Fig. 2 illustrates how such mistakes are eliminated when conidial ornamentation is employed.

I also referred earlier to the misplaced reliance attached to differences in ascospore topography as revealed by the light microscope. Fig.3 (b and d) shows that even when their form is examined using the SEM, differences are sometimes barely discernible. The ascospore topography of *Aspergillus repens, Aspergillus ruber* and *Aspergillus amstelodami* grade one into another. The ascospore of *A.amstelodami* is distinct from that of the other two but differentiation between the ascospores of *A.repens* and *A.ruber* is doubtful. Therefore, the credibility of light microscope diagnoses using these ascospores must be in question. Interestingly mycologists readily accept that light microscopy is inadequate for differentiating conidial topography. However, scanning electron micrographs of the conidia of these same three species show that conidial characteristics are superior to those of the ascospore for their identification (Fig. 3a, c and e).

I now turn to specific cases where determinations involving mycotoxin producing species have been based upon misidentifications made by mycologists with high levels of expertise. The first concerns the two important species *Aspergillus flavus* and *Aspergillus parasiticus*. Isolates of these two species are maintained in all the major world collections and are used extensively for reference and as verified isolates for mycotoxin research projects. The integrity of isolate labels associated with such collections is rarely if ever questioned.

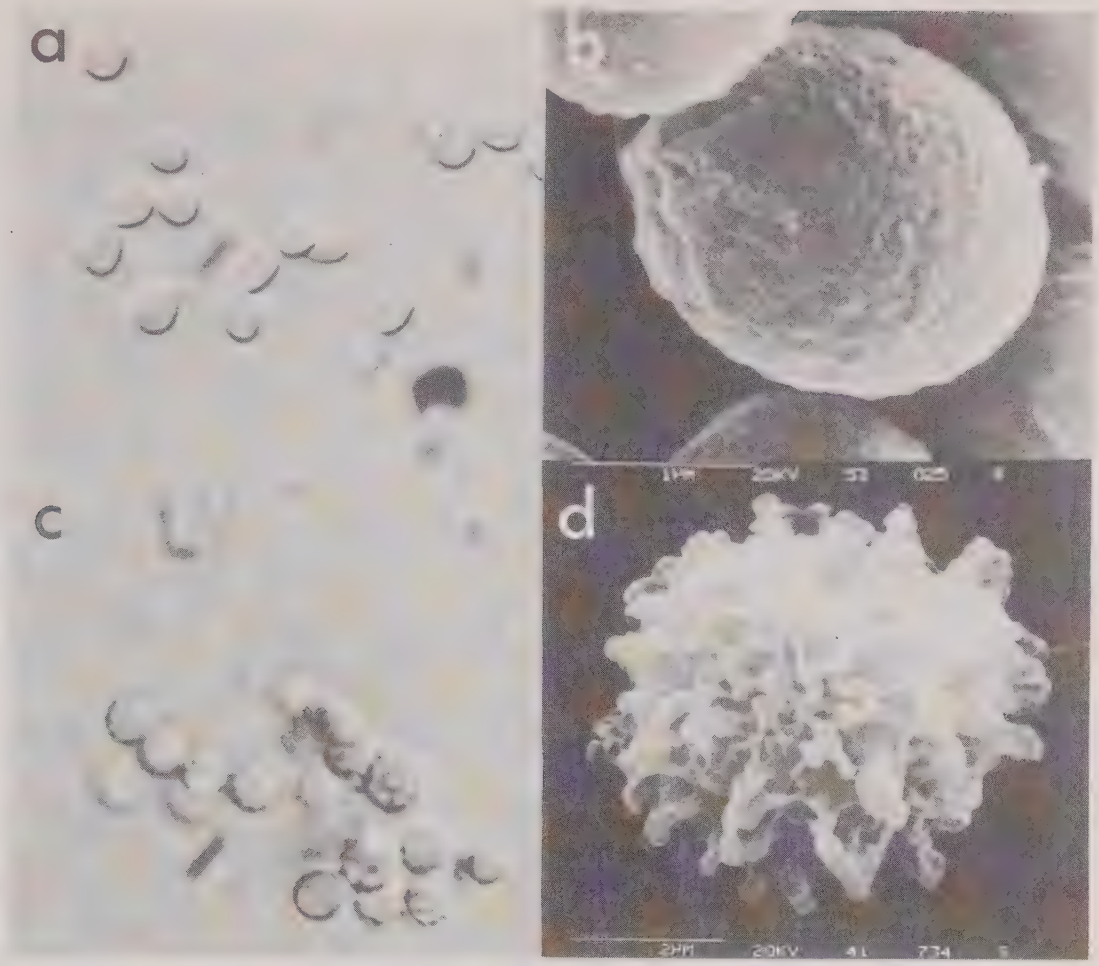

Fig. 1. Conidial ornamentation in *A. avenaceus* (a & b) and *A. terricola* (c & d) as revealed by light and scanning electron microscopy respectively, illustrating the superior resolving power of the latter.

One aspect of my study on intraspecific variability involved examination of a large number of *A.parasiticus* isolates which I obtained from numerous world collections (Kozakiewicz 1982). This species was originally described by Speare in Hawaii (Speare 1912) from material on Hawaiian sugar cane. Subsequently his original isolate was subcultured around the world. SEM examination of all extant isolates revealed conidia of two distinct morphs. Isolates always consisted of one or other of these two morphs but never mixtures of both.

When these morphs are attributed to their respective cultures, a sharp dichotomy is revealed. One form occurs in the ex-type isolate of *A.parasiticus* (Fig. 4b) whilst the other has been established as that of *A.flavus* (Fig. 5b). Thus, use of this new technique revealed that ex-type cultures of *A.parasiticus* held in three major world collections are in fact *A.flavus*. A similar situation has also been shown to occur in other world centres. Ten per cent of the *A.parasiticus* cultures I have examined represent misidentifications of *A.flavus*. Whilst, in respect of *A.flavus*, 25 per cent of the cultures are actually *A. parasiticus*. The use of conidial ornamentation would eliminate such mistakes.

A second case of misidentification resulted in a published statement attributing aflatoxin production to an isolate of *Aspergillus oryzae* (El-Hag & Morse 1976), a species widely used in the oriental food manufacturing industry. A protracted published argument ensued between the authors of this statement and the curator of the donor culture collection (Fennell 1976).

On subsequent examination of a sample returned to the donor institute the curator, a world authority, declared the returned sample to be a pure culture of *A. parasiticus* heavily infested with mites, thus accounting for the aflatoxin production. Nevertheless, the argument remained unsolved and continued to appear in the literature (Morse 1976), the mycotoxin researchers insisting they were working with a mycotoxin producing variant of *A. oryzae*. The question remained open until SEM examination of an isolate grown from the batch culture in dispute revealed the presence of two conidial forms. One I identified as *A. parasiticus*, the other as *A.oryzae*. Thus, using this technique, the ability to declare the isolate as mixed entirely solves the question (Fig. 4).

Fig. 2 Illustrating the use of conidial ornamentation to positively identify species which may be misidentified through the possession of the same colony colour.
Black or brown-black colonies: a) *A. niger*, c) *A. phoenicis* d) *A. carbonarius*, b) *A. ustus*.
White colonies: e) *A. candidus*, f) *A. niveoglaucus*.
(NB Ornamentation also indicates *A. niger* and *A. phoenicis* to be conspecific)

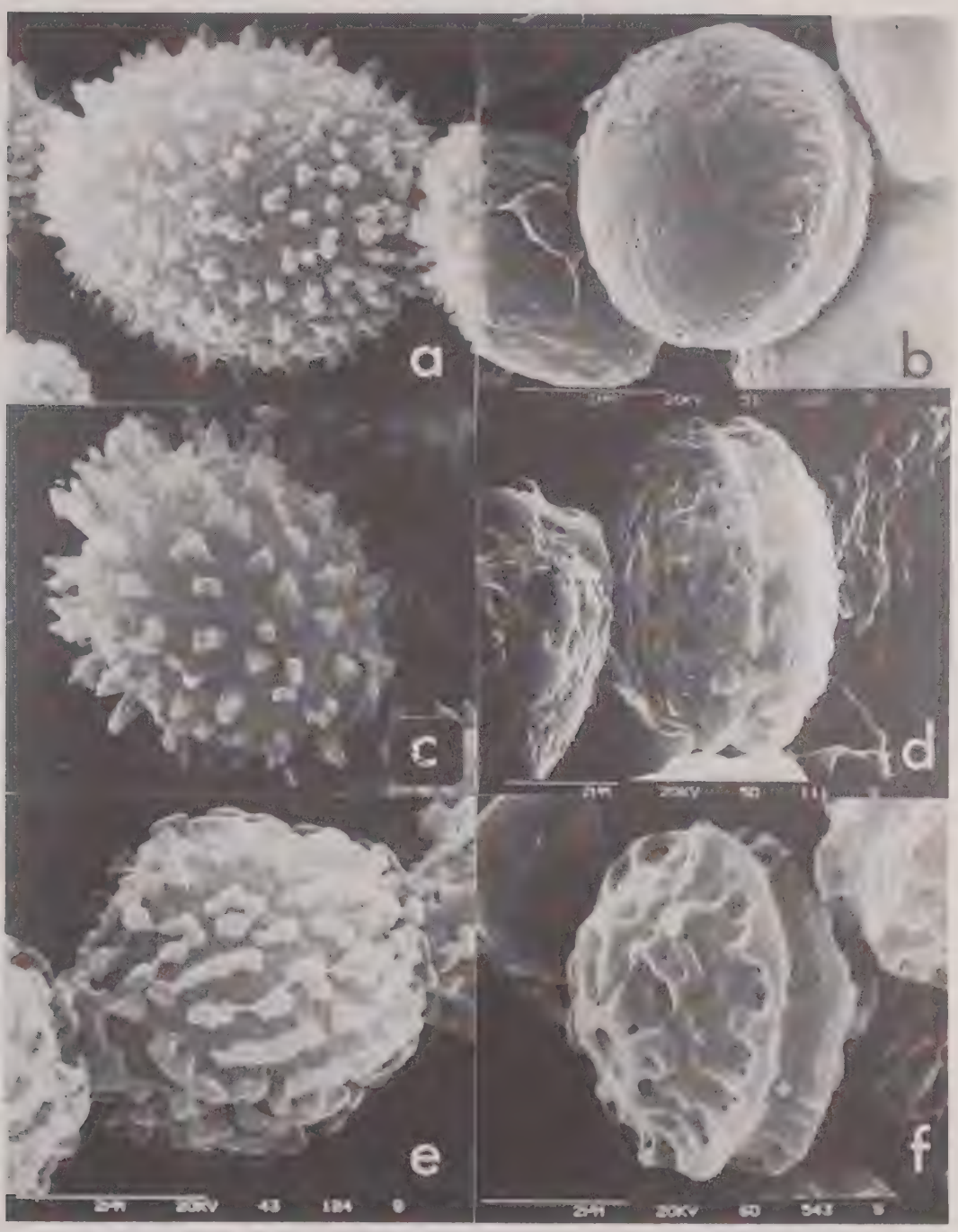

Fig. 3 SEM micrographs illustrating the superiority of conidial ornamentation (left series) over ascospore ornamentation (right series) for identification of *A.repens* (a,b), *A.ruber* (c,d) and *A.amstelodami* (e,f).

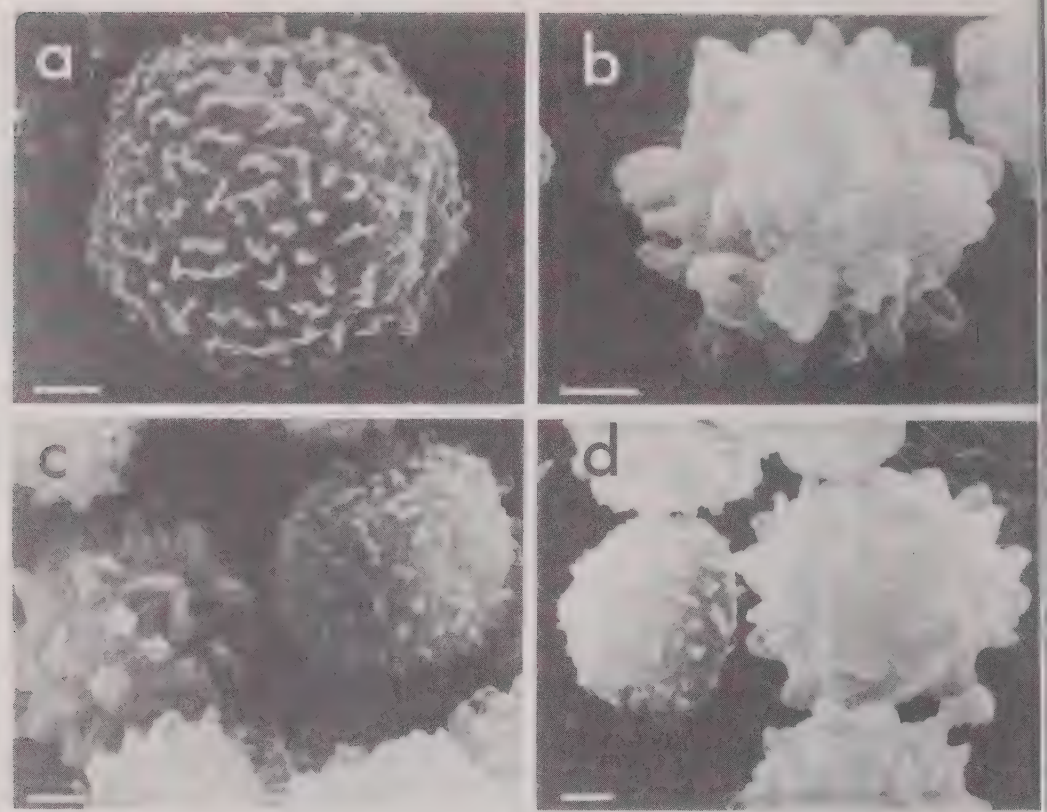

Fig. 4. Conidial ornamentation of *A. oryzae* (a) and *A. parasiticus* (b) illustrating how the differences instantly identify contaminated cultures not discernible by visual or light microscopy. Contaminated *A. oryzae* cultures are (c) NRRL 1988 and (d) ATCC 9362. Scale indicates one μm.

Finally, a hidden but important bonus is associated with this new approach. The need for speedy identifications is obvious, yet traditional methods require the employment of direct and dilution plating techniques. These are time consuming and labour intensive occupations, which to a large extent we should now be able to eliminate. The freely sporulating nature of this genus means that an abundance of conidia are present on the commodity, within its interstitial spaces as well as in the store atmosphere. Two techniques are being developed to exploit this situation.

Firstly, it is possible to identify inoculum by direct examination in the SEM of representative samples of the infected material, e.g. individual cereal kernels. Alternatively, a minor adaptation of a Cambridge Scientific Instruments specimen stub (developed for examination of fabric weaves) enables one to sample interstitial spaces or the atmosphere and directly examine the results within the SEM. The shaft of the stub is drilled through, a suitable filter is attached to its face and the shaft attached to a suction pump allowing a monitored amount of atmosphere to be sucked past the filter. The distinct morphoforms of six species commonly associated with stored cereals illustrated in Fig. 5, demonstrate the usefulness of this technique.

The development of a series of SEMs for routine use in a variety of institutes and industries means that a suitable model is financially within the reach of most organisations. The technique is taxonomically reliable giving an accurate and speedy identification of the economically important *Aspergillus* species. The implications of current misidentifications applied to isolates used in mycotoxin research compared with the accuracy of identification implicit in this technique cannot be ignored.

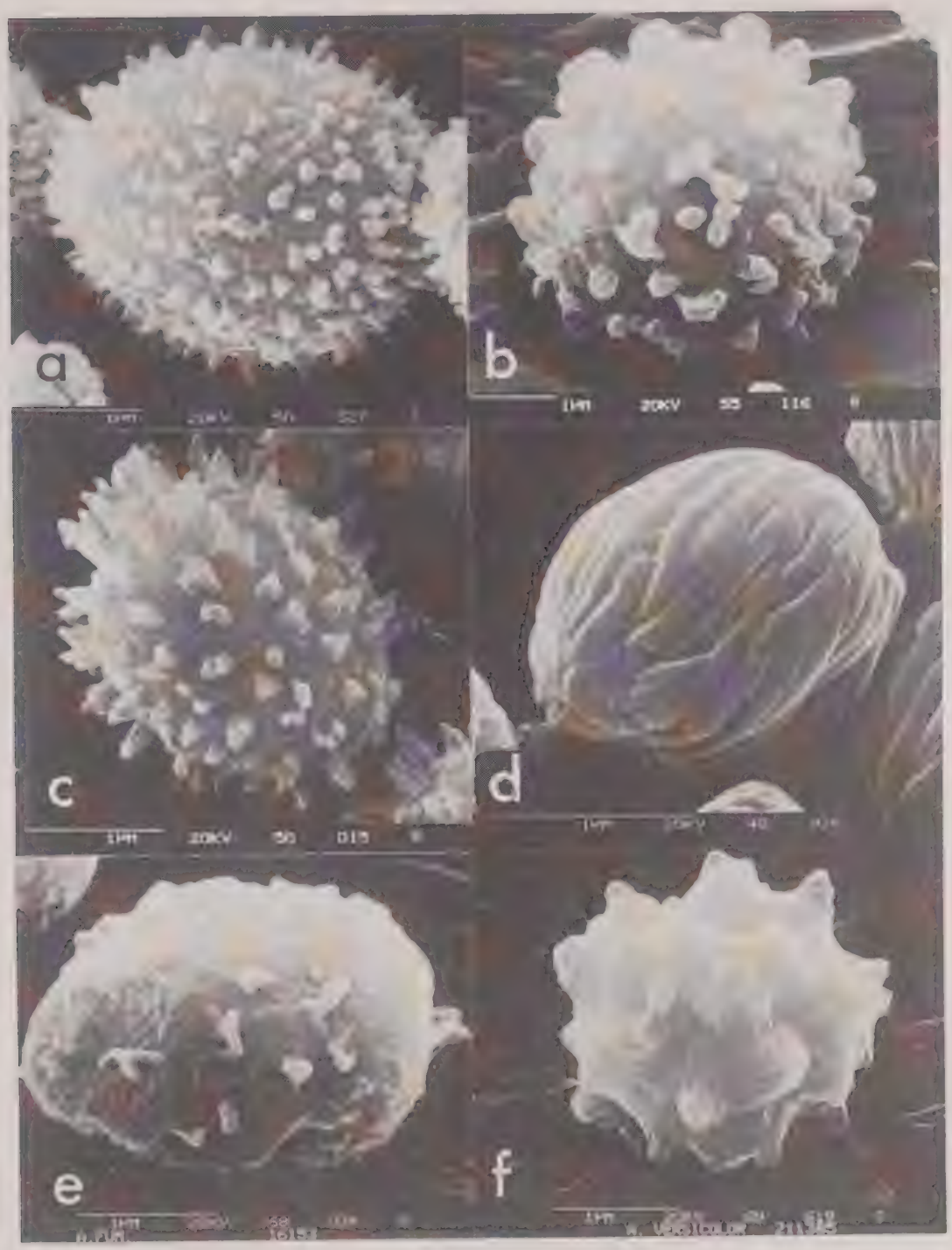

Fig. 5 SEM micrographs illustrating differences in conidial ornamentation exhibited by six common stored product *Aspergillus* species: a) *A. repens*, b) *A. flavus*, c) *A. ruber*, d) *A. terreus*, e) A. *fumigatus*, f) *A. versicolor*. Each topography is distinct and unique to one of the species.

References

El-Hag, N.; Morse, R.E. (1976) Aflatoxin production by a variant of *Aspergillus oryzae* (NRRL 1988) on cowpeas (*Vigna sinensis*). *Science* **192**, 1345-1346.

Fennell, D.I. (1976) *Aspergillus oryzae* (NRRL 1988): A clarificaton. *Science* **194**, 1188.

Kozakiewicz, Z. (1982) The identity and typification of *Aspergillus parasiticus*. *Mycotaxon* **15**, 293-305.

Kozakiewicz, Z. (1983) PhD thesis, University of London.

Malloch, D.; Cain, R.F. (1972) New species and combinations of cleistothecial Ascomycetes. *Canadian Journal of Botany* **50**, (1), 61-72.

Morse, R.E. (1976) *Aspergillus oryzae* (NRRL 1988): A clarification. *Science* **194**, 1188.

Raper, K.B.; Fennell, D.I. (1965) *The genus Aspergillus*. Baltimore; Williams & Wilkins.

Speare, A.T. (1912) Fungi parasitic upon insects injurious to sugar cane. *Hawaiian Sugar Planters Association Experimental Station Pathology and Physiology Series Bulletin* **12**, 1-62.

Subramanian, C.V. (1972) The perfect states of *Aspergillus*. *Current Science* **41**, 755-761.

Thom, C.; Church, M.B. (1926) *The Aspergilli*. Baltimore; Williams & Wilkins.

Thom, C.; Raper, K.B. (1945) *Manual of the Aspergilli*. Baltimore; Williams & Wilkins.

Spoilage and Mycotoxins of Cereals and other Stored Products.
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Immunological Detection of Fungi in Plants, Including Stored Cereals

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I. Summary

The literature on the use of immunological techniques to attempt to detect fungi, mostly plant pathogens, in plant tissues and related substrates is reviewed. About an equal number of papers have been written in which plant pathogenic or saprophytic fungi were investigated immunologically but the study was not followed through to the plant itself. Very few instances of their application to stored cereals have been found.

II. Introduction

Immunology, the study of the immune system and its associated antibodies, is of central importance in medicine. Plants do not produce proteinaceous antibodies when they are infested with pathogenic organisms. However, it has been suggested (Tarr 1972) that phytoalexins are somewhat analogous to animal antibodies, but they are mostly restricted to the site of infection and are relatively simple compounds. Many substances can elicit an immune response after injection into an animal. Antibody-containing serum or 'antiserum' can be obtained and used in a variety of immunoassay techniques to detect the original or 'homologous' antigen. Because the vegetative or metabolically active part of a fungus, the mycelium, looks very similar in nearly all fungi, it is probably only by the use of immunological techniques that such nondescript material can be detected, identified and quantified. Furthermore, mycelium is the part of most interest when we are concerned with biodeterioration and mycotoxin production by fungi in stored products (Clarke 1968).

Before 1975 all antibodies used to detect fungi as antigens were derived from the serum of animals immunised with a suitable antigen. In 1975 Köhler and Milstein published a revolutionary new technique by which 'pure' antibodies were produced by tissue culture in which hybrid cells (hybridomas) were derived from fusing immunised murine spleen cells with non-antibody producing murine malignant myeloma cells. The former type of antibody is now termed polyclonal and the latter monoclonal. While polyclonal antibodies have been widely used for detecting fungi in plant tissue, monoclonal antibodies have hardly ever been used for this purpose. However, both types of

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antibody have been used successfully to quantify mycotoxins, as described in the paper of Kemp and Morgan, which follows this.

Preece (1971) presented a relatively short review on immunological techniques in mycology after an earlier shorter review (Preece 1968) on the use of the fluorescent antibody technique for identifying plant pathogenic fungi, as did Hornok (1975). A very useful and comprehensive review was presented by Pepys & Longbottom (1978) on immunological techniques for fungi but it confined itself to medically important fungi; nevertheless many of their comments will apply to plant pathogenic or saprophytic fungi. Clark (1981) provided an excellent review of immunosorbent assays in plant pathology but dealt chiefly with the use of the enzyme-linked immunosorbent assay (ELISA) for detecting plant pathogenic viruses.

Therefore, this paper is an up-to-date review on the use of immunological techniques for detecting fungi in plants.

1. Form of antigen

There are almost as many ways of producing fungal antigens as papers on the subject, with little attempt at any standardisation. However, certain general features emerge.

Mycelium has been the most commonly used form. It is produced by shake or stationary culture in a liquid medium. Media used have varied greatly but the one used most often for plant studies and by ourselves is that of Burrell *et al.* (1966). The mycelium is then usually homogenised in phosphate-buffered saline (PBS) and centrifuged. The pellet of homogenised mycelium is then often called a 'cell wall' antigen and the supernatant is termed the 'soluble' antigen. Many workers have stated amount of mycelium in the preparation, as for instance 25 mg dry weight/ml (Wyatt & di Menna 1981). Very few workers, e.g. White (1976), have stated the amount of soluble protein, which is more meaningful. We have developed (Clarke *et al.*, unpublished) a microplate modification of the Coomassie blue protein estimation method of Bradford (1976).

Instead of mycelium, whole spores of plant pathogenic fungi can be used, especially species that grow poorly or not at all in culture, e.g. smuts (Banowetz *et al.* 1984).

Penicillium viridicatum was chosen as our first antigen as it is a common species of stored grain and strains of it can produce the mycotoxin ochratoxin A.

2. Host animal and immunisation

In the vast majority of cases the rabbit has been the chosen animal for raising polyclonal antibodies against fungal antigens. Normally several intramuscular injections are given over a number of weeks. If a 'soluble' form of antigen is being used, it is considered necessary to incorporate it in Freund's Complete Adjuvant (Herbert 1978). For later injections the antigen can be used in Freund's Incomplete Adjuvant, and an aqueous antigen without any adjuvant is often given as a final booster dose. Many workers, including ourselves, have carried out successful immunisations without adjuvants using a particulate 'cell wall' preparation of fungi. In the few studies raising monoclonal antibodies to fungi, mice of the BALB/c and C57 BL/6J strains have been exclusively used. Apart from the fact that much smaller amounts (usually 0.1 ml) of antigen are injected by the intraperitoneal route, immunisation protocols are generally similar to those for the rabbit. In our study, serum was obtained by the method of Goding (1983).

3. Hybridoma protocol

Since the initial paper by Köhler & Milstein (1975), countless modifications have been made by other workers to their basic protocol. The various aspects of the hybridoma fusion technique are well discussed by Goding (1983). Our own technique, like that of most workers, is a combination of recommendations from several sources and will be given as a general guide.

A spleen was removed aseptically and a cell suspension was made in serum-free RPMI 1640 tissue culture medium (Moore *et al.* 1967). The lymphocytes were then mixed in the ratio of 10:1 with healthy, rapidly growing cells of the P3. X63. Ag 8. 653 non-immunoglobulin producing mouse myeloma line (Kearney *et al.* 1979). Polyethylene glycol (50%), molecular weight 4000 (Pontecorvo 1976), was added to bring about heterokaryon formation and subsequent nuclear fusion at the next cell division. Hybrid cells were plated variously into either 24-well dishes or 96-well microplates, with or without feeder cells (Zola & Brooks 1982) and with or without myeloma cell supernatant (Løvborg 1982). Cell cultures were incubated in a CO_2 incubator at 37°C, 99% relative humidity and in an atmosphere of 5% CO_2/95% air. After 16-24 h (Oi & Herzenberg 1980) a solution of hypoxanthine/aminopterin/thymidine, or HAT (Littlefield 1964) was added to kill unfused myeloma cells. After 2 weeks hypoxanthine/thymidine was added to be replaced a week later by normal, complete RPMI 1640. Hybridoma colonies were first observed 9 days after fusion. Whenever the hybridoma colonies were fed, supernatants were removed aseptically and tested for antibody by an indirect enzyme-linked immunosorbent assay or ELISA (Engvall & Perlmann 1971; Weeman & Schuurs 1971), which is discussed in more detail below. Cells from wells that appeared positive

from one or more ELISAs were frozen in complete RPMI 1640 containing 10% dimethylsulphoxide as cryoprotectant, then stored in vapour-phase liquid nitrogen (c. -140°C). After rapid thawing at 37°C, hybrid cells were cloned by limiting dilution at 5.0, 1.0 and 0.5 cells per microplate well (Zola & Brooks 1982). If less than 63% of wells yielded colonies, it was assumed statistically (Goding 1983) that they were single colonies or 'clones'; confirmation of this was also checked microscopically.

4. Enzyme-linked immunosorbent assay (ELISA)

Our technique was based largely on the indirect ELISA for antibody (Voller *et al.* 1979). Soluble *Penicillium viridicatum* (strain PIL 333) antigen (0.5-10 μg/ml soluble protein) was coated overnight at 4°C. After three washings with phosphate-buffered saline - Tween 20 (PBST), either dilutions from 1/1000 to 1/100,000 of hyperimmune mouse serum or undiluted supernatants of the mouse-mouse hybridomas were added. After 2 h incubation at 25°C and three further PBST washings, a 1/5000 dilution of horseradish peroxidase-conjugated goat anti-mouse polyspecific immunoglobulins was incubated in the wells for 3 h at 25°C. After three further PBST washings, the substrate consisting of hydrogen peroxide/orthophenylene diamine was added and the reaction stopped with 2.5M sulphuric acid, normally after 30 min. Absorbances were read at dual wavelengths of 490 nm/630 nm using a Dynatech MR600 spectrophotometer, controlled by an Apple IIe microcomputer using the Dynatech ELISA/MEAN programme and printouts were obtained on an EPSON FX-80 printer.

The technique just described is essentially for measurement of antibodies. If measurement of antigens in, say, plant tissue is the aim, slightly different ELISA techniques have to be used, for which the publications of Voller *et al.* (1979), Clark (1981) and Johnson *et al.* (1982) should be consulted.

5. Immunofluorescence assay

Immunofluorescence is basically very similar to ELISA except the second antibody in the indirect system is conjugated to a fluorochrome, the most commonly used fluorochrome being fluorescein isothiocyanate (FITC). When excited by a UV light source, it emits a bright green fluorescence at around 510 nm, which can be viewed with a microscope. It must be emphasised that the above technique is related to the original 'fluorescent antibody' technique of Coons *et al.* (1941). There now exist much more quantitative fluoroimmunoassay (FIA) and immunofluorimetric (FMA) techniques (Maggio 1980) which have similar performance to radioimmunoassay (Yalow & Berson 1959). However, there appear to be no reports in the literature of these assays being used for detection of fungal antigens in plant tissue.

While there has been very little work on the use of immunological techniques to detect fungi on stored cereals and our work is at a relatively early stage, it would seem appropriate to review the use that has been made of such techniques for detecting fungi in other plants, if only to indicate the great potential that they offer for studies on stored grain or other stored products.

III. Detection of fungi in plants and certain other substrates such as soil and litter

The papers in Table 1 are those in which antibodies were raised against a plant pathogenic or saprophytic fungus and these antibodies were then used to try to detect the fungus in the host plant. No claim is made that every paper on the subject has been noted but a concerted effort was made to achieve as full a cover as possible. Schmidt & Bankole (1962) were the first to use immunological techniques in this way, though only with *Aspergillus flavus* in sterile soil. It is interesting that cereals and grasses, trees and soil are predominant hosts or substrates studied.

The majority of authors have used the polyclonal antibody approach. Only Benhamou *et al.* (1984) successfully used monoclonal antibodies in their studies on the Dutch Elm Disease pathogen. Direct or indirect immunofluorescence was the most commonly used assay technique. Many authors tested the degree of cross-reactivity of their antisera against either closely related species of the same genus or clearly unrelated fungi that could occur in the same environment as the pathogen. The standard way of reducing cross-reaction with a polyclonal antiserum is to cross-absorb it, that is to mix it with the 'heterologous' cross-reacting antigen(s) thereby absorbing out the fractions that gave such unwanted cross reactions. However Holland & Choo (1970) reported that after carrying out cross-absorption their antiserum no longer reacted positively with the homologous antigen, *Ophiobolus Gaeumannomyces graminis*. This is an argument in favour of monclonal antibodies. While they can also exhibit cross-reaction with heterologous antigens, screening should lead to hybridomas being found which react only with the antigenic determinant of choice.

Concerning the very small amount of work on stored cereals, Warnock (1971) used an indirect immunofluoresence technique to estimate the amount of *Penicilium cyclopium* in stored barley

Table 1. *Immunological detection of fungi actually on or in plants or related substrates*

Plant or other substrate	Fungus (used for raising antibodies)	PAb or MAb	IA technique	Degree of success	Authors	Year
Cereals including grasses						
Barley	*Penicillium viridicatum*	PAb & MAb	indirect ELISA	partial	Clarke *et al.*	herein
Barley	*P. cyclopium*	PAb	indirect IF	yes	Warnock	1971
Barley	*Alternaria alternata, Aspergillus flavus, P. cyclopium*	PAb	indirect IF	yes	Warnock	1973
Tall fescue	*Epichloe typhina*	PAb	indirect ELISA	yes	Johnson *et al.*	1982
Maize	*Fusarium culmorum*	PAb	direct & indirect IF		Hornok & Jagicza	1973
Maize	*F. moniliforme*	PAb	ID & sucrose gradient analysis	yes	Marshall & Partridge	1981
Maize	*Pythium graminicola*	PAb	direct IF	some	White	1976
Oats & wheat	*Ophiobolus graminis*	PAb	direct & indirect IF	yes	Choo & Holland	1970
Other herbaceous agricultural plants						
Clover	*Sclerotinia trifoliorum*	PAb	indirect ELISA	yes	Scott	1981a
Tomato	*Pythium aphanidermatum, Phytophthora parasitica, Ph. parasitica* var. *nicotianae*	PAb	ID	partial	Morton & Dukes	1967
Shrubs & trees						
Elm	*Ophiostoma (Ceratocystis) ulmi* .	MAb	indirect IF indirect immuno-peroxidase	yes	Benhamou *et al.* (abstract only)	1984
Elm	*O. ulmi*	PAb	indirect IF indirect immuno-peroxidase	yes	Elgersma & Steerenberg	1978
Elm	*O. ulmi*	PAb	indirect ELISA	yes	Scheffer & Elgersma	1981
Pine	*Polyporus tomentosus*	PAb	direct IF	yes	Kumar & Patton (abstract only)	1964
Pine	mycorrhizal fungi (*Pisolithus tinctorius, Thelephora terrestris)*	PAb	direct IF	yes	Schmidt *et al.*	1974
Leaf litter						
Oak leaf litter	*Mycena galopus*	PAb	indirect IF	partial	Frankland *et al.*	1981
Soil						
Soil	*Phytophthora megasperma, Ph. cinnamoni*	PAb	indirect IF	partial	MacDonald & Duniway	1979
Soil	*Phytophthora cinnamoni*	PAb	direct IF	partial	Malajczuk *et al.*	1978
Soil	*Phytophthora cinnamoni*	PAb	direct & indirect IF	partial	Malajczuk *et al.*	1975
Soil (sterile only)	*Aspergillus flavus*	PAb	direct IF	partial	Schmidt & Bankole	1962, 1963 & 1965
Soil	*Penicillium crustosum, P. janczewskii*	PAb	indirect IF	yes	Wyatt & Di Menna	1981

Key to abbreviations: PAb= polyclonal antibody; MAb= monoclonal antibody; IA= immunoassay; IF= immunofluorescence; ID= immunodiffusion; IE= immunoelectrophoresis.

Table 2. *Immunological studies on plant pathogens or saprophytic fungi not followed through to the plant*

Author	Year	Source plant	Fungus used for raising antibodies	PAb or MAb	IA techniques	Purpose
Abd-El-Rehim & Fadel	1980	—	*Fusarium* spp. *(F. culmorum, F. moniliforme, F. oxysporum, F. semitectum, F. solani)*	PAb	IE	taxonomic
Amos & Burrell	1967	Oak	*Ceratocystis* spp. (7)	PAb	ID, indirect IF	taxonomic
Anathanarayanan & Reddy	1984	arecanut palm	*Ganoderma lucidum*	PAb	tube precipitation	detection
Banowetz *et al.*	1984	wheat	*Tilletia caries, T. controversa*	MAb	ID, indirect IF, IE, ELISA	detection
Beno & Allen	1964	sorghum	*Puccinia sorghi*	PAb	direct IF	detection
Burrell *et al.*	1966	—	*Phytophthora* spp. *(P. cactorum, P. cinnamoni, P. erythroseptica)*	PAb	ID, indirect IF	taxonomic
Chard *et al.*	1983	leaf litter	*Mycena galopus*	PAb	ID, IE	taxonomic
Fitzell *et al.*	1980	many plants	*Verticillium* spp. *(V. alboatrum, V. dahliae, V. tricorpus, V. nigrescens*	PAb	ID, IE, direct IF	taxonomic
Fulcher & Holland	1971	(cereal)	*Ophiobolus graminis*	PAb	indirect IF	detection
Halsall	1976	—	*Phytophthora* spp. *(P. cinnamoni, P. drechsleri, P. cryptogea, P. cambivora)* *Pythium irregulare, P. acanthicum*	PAb	ID, agglutination, indirect IF	taxonomic
Holland & Choo	1970	wheat & oats	*Ophiobolus graminis*	PAb	IE	detection
Iannelli *et al.*	1982	various	*Fusarium oxysporum, F. moniliforme*	PAb	ID	taxonomic
Merz *et al.*	1969	—	*Phytophthora* spp. *(P. cinnamoni, P. arecae, P. citrophthora, P. palmivora, P. meadii)*	PAb	ID, indirect IF	taxonomic
Miyazaki *et al.*	1979	—	*Absidia cylindrospora* (polysaccharide preparation)	PAb	precipitation, inhibition	mainly biochemical
Mohan & Ride	1982	hop	*Verticillium albo-atrum*	PAb	IE (counter & rocket line)	to explain pathogenicity
Mohan & Ride	1983	hop	*V. albo-atrum*	PAb	indirect IF, IE (rocket line)	to explain virulence
Preece & Cooper	1969	strawberry	*Botrytis cinerea*	PAb	agglutination, direct & indirect IF	detection
Rappuoli *et al.*	1982	—	*Fusarium graminearum*	PAb	ID, agglutination	detection
Scott	1981b	clover	*Sclerotinia* spp. *(S. trifoliorum, S. sclerotiorum, S. minor)*	PAb	ID, IE (crossed)	taxonomic
Severini *et al.*	1982	—	*Fusarium graminearum*	PAb	ELISA (done in tubes)	detection
Wyllie & DeVay	1970	cotton	*Verticillium albo-atrum, V. nigrescens*	PAb	agglutination, ID, IE	to explain virulence

naturally contaminated with this species and with other common storage fungi of the genus *Aspergillus*. He claimed to have had no cross-reactivity of the anti-*Penicillium* rabbit antiserum. In a later study, Warnock (1973) extended the work by raising polyclonal antibodies against *Alternaria alternata*, *Aspergillus flavus* and *P. cyclopium*. An indirect immunofluorescence with pure cultures as antigens revealed that the anti-*A. flavus* antiserum did cross-react with *A. candidus* and *A. fumigatus*; the anti-*P. cyclopium* antiserum cross-reacted with *P. brefeldianium* and *P. expansum*. Little cross-reaction occurred between these antisera and heterologous genera. When immunofluorescence was used with naturally contaminated grain as antigen, there was good agreement with direct plating onto agar for *Aspergillus* and *Penicillium* but not for *Alternaria*.

In our own work we have raised monoclonal (hybridoma) antibodies against *Penicillium viridicatum*, but have not yet used them with a grain substrate. We have, however, recently conducted an indirect ELISA using a polyclonal mouse antibody in which grain inoculated with the homologous *Penicillium* was compared with uninoculated grain. We were able to differentiate between the two in homogenate supernatants either undiluted or diluted up to 1:1000 in coating buffer. This is a most encouraging result, for such an immunoassay is much more quantitative than the immunofluorescence used by Warnock.

A number of papers roughly equal to that in Table 1 is listed in Table 2, where fungi were again investigated immunologically but, for one reason or another, the authors did not attempt to detect fungal contamination of the plant tissue. Some papers had the elucidation of taxonomic relationships as their main aim, a topic not being covered here.

IV. Concluding discussion

Two general points emerge from this review of the literature. The first is that immunological techniques offer great potential. The second is that such reagents must be proved to be reliable if they are to supplant existing, more laborious methods. But why is it that so relatively few immunological studies of fungi in or on plants have been made? Unlike the medical mycologist, the plant pathologist is not provided with a ready-made source of antibodies and must raise them in animals for the specific antigen interest. It has been suggested (Burrell *et al.* 1966) that fungi may not be very good antigenic material or that they are more antigenically complex and therefore difficult to study compared with viruses and bacteria (Hornok 1975). But Preece (1971) suggested that many mycologists regarded immunological techniques as being part of other more difficult disciplines. That was written when only polyclonal antibodies were being used. Raising monoclonal antibodies must involve in addition a knowledge of animal cell culture and use of more sensitive, essentially biochemical immunoassay techniques, making Preece's contention even more pertinent.

Fungi and monoclonal antibodies are included in the flourishing area of biotechnology. It now seems highly likely that biochemical and immunological studies on fungi will flourish in the future, helping to unravel their complex antigenic nature. Such studies are bound to yield dividends in the more applied areas, such as prevention of fungal growth in stored cereals.

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References

Abd-El-Rehim, M.A.; Fadel, F.M. (1980) Immunoelectrophoretic differences between phytopathogenic *Fusarium* species. *Biochemical Systematics and Ecology* **8**, 7-9.

Amos, R.E.; Burrell, R.G. (1967) Serological differentiation in *Ceratocystis*. *Phytopathology* **57**, 32-34.

Ananthanarayanan, T.V.; Reddy, M.K. (1984) Serological test for the diagnosis of *Ganoderma lucidum*. *Current Science* **53**, 97-98.

Banowetz, G.M.; Trione, E.J.; Krygier, B.B. (1984) Immunological comparison of teliospores of two wheat bunt fungi, *Tilletia* species, using monoclonal antibodies and antisera. *Mycologia* **76**, 51-62.

Benhamou, N.; Ouellette, G.B.; Lafontaine, J.-G.; Joly, J.R. (1984) Monoclonal antibodies to a glycopeptide produced by *Ophiostoma ulmi*. *Canadian Journal of Plant Pathology* **6**, 260.

Beno, D.W.; Allen, O.N. (1964) Immunofluorescent staining for the identification of *Puccinia sorghi* germinated uredospores. *Phytopathology* **54**, 872-873.

Bradford, M.M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* **72**, 248-254.

rrell, R.G.; Clayton, C.W.; Gallegly, M.E.; Lilly, V.G. (1966) Factors affecting the antigenicity of the mycelium in three species of *Phytophthora*. *Phytopathology* **56**, 422-426.

nard, J.M.; Gray, T.R.G.; Frankland, J.C. (1983) Antigenicity of *Mycena galopus*. *Translations of the British Mycological Society* **81**, 503-511.

hoo, Y.S.; Holland, A.A. (1970) Direct and indirect fluorescent antibody staining of *Ophiobolus graminis* Sacc. in culture and in the rhizosphore of cereal plants. *Antonie van Leeuwenhoek* **36**, 549-554.

lark, M.F. (1981) Immunosorbent assays in plant pathology. *Annual Review of Phytopathology* **19**, 83-106.

larke, J.H. (1968) Fungi in stored products. *Tropical Stored Products Information* **15**, 3-14.

oons, A.H.; Creech, H.J.; Jones, R.N. (1941) Immunological properties of an antibody containing a fluorescent group. *Proceedings of the Society for Experimental Biology (New York)* **47**, 200-202.

lgersma, D.M.; Steerenberg, P.A. (1978) A study of the development of *Ophiostoma ulmi* in elms with immunological techniques. *Netherlands Journal of Plant Pathology* **84**, 127-133.

ngvall, E.; Perlmann, P. (1971) Enzyme-linked immunosorbent assay (ELISA). Quantitative assay of immunoglobulin G. *Immunochemistry* **8**, 871-874.

itzell, R.; Fahy, P.C.; Evans, G. (1980) Serological studies of some Australian isolates of *Verticillium* spp. *Australian Journal of Biological Science* **33**, 115-124.

rankland, J.C.; Bailey, A.D.; Gray, T.R.G.; Holland, A.A. (1981) Development of an immunological technique for estimating mycelial biomass of *Mycena galopus* in leaf litter. *Soil Biology and Biochemistry* **13**, 87-92.

ulcher, R.G.; Holland, A.A. (1971) Fluorescent antibody staining of 1-2 μm sections of hyphae of *Ophiobolus graminis* Sacc. embedded in glycol methacrylate. *Archiv für Mikrobiologie* **75**, 281-284.

oding, J.W. (1983) *Monoclonal antibodies: principles and practice*. London & New York; Academic Press.

Halsall, D.M. (1976) Specificity of cytoplasmic and cell-wall antigens from four species of *Phytophthora*. *Journal of General Microbiology* **94**, 149-158.

Herbert, W.J. (1978) Mineral-oil adjuvants and the immunization of laboratory animals. In *Handbook of Experimental Immunology*. Vol 3. *Application of immunological methods*. (D.M. Weir, ed.), A3.1-A3.15. Oxford; Blackwell Scientific Publications.

Holland, A.A.; Choo, Y.S. (1970) Immunoelectrophoretic characteristics of *Ophiobolus graminis* Sacc. as an aid in classification and determination. *Antonie van Leeuwenhoek* **36**, 541-548.

Hornok, L. (1975) Immunofluoreszeencia, egy uj modszer a novenykorokozo gombak azonositasara. *Novenytermeles* **24**, 181-184.

Hornok, L.; Jagicza, A. (1973) Fluorescent antibody staining of *Fusarium culmorum*. *Acta Phytopathologica Academiae Scientiarum Hungaricae* **8**, 357-363.

Iannelli, D.; Capparelli, R.; Cristinzio, G.; Marziano, F.; Scala, F.; Noviello, G. (1982) Serological differentiation among *formae speciales* and physiological races of *Fusarium oxysporum*. *Mycologia* **74** 313-319.

Johnson, M.C.; Pirone, T.P.; Siegel, M.R.; Varney, D.R. (1982) Detection of *Epichloe typhina* in tall fescue by means of enzyme-linked immunosorbent assay. *Phytopathology* **72**, 647-650.

Kearney, J.F.; Radbruch, A.; Liesegang, B.; Rajewsky, K. (1979) A new mouse myeloma cell line which has lost immunoglobulin expression but permits the construction of antibody in secreting hybrid cell lines. *Journal of Immunology* **123**, 1548-1550.

Köhler, G.; Milstein, C. (1975) Continuous culture of fused cells secreting antibody of predefined specificity. *Nature, London* **256**, 495-497.

Kumar, D.; Patton, R.F. (1964) Fluorescent antibody technique for detection of *Polyporus tomentosus*. *Phytopathology* **54**, 898.

Littlefield, J.W. (1964) Selection of hybrids from matings of fibroblasts *in vitro* and their presumed recombinants. *Science* **145**, 709-710.

Løvborg, U. (1982) *Monoclonal antibodies: production & maintenance*. London; William Heinemann Medical Books.

MacDonald, J.D.; Duniway, J.M. (1979) Use of fluorescent antibodies to study the survival of *Phytophthora megasperma* and *P.cinnamomi* zoospores in soil. *Phytopathology* **69**, 436-441.

Maggio, E.T. (1980) Recent advances in heterogeneous fluorescence immunoassays. In *Immunoassays: Clinical Laboratory Techniques for the 1980s*. R.K. Nakamura; W.R. Dito; E.S. Tucker III, eds), 1-12. New York; Alan R. Liss Inc.

Malajczuk, N.; Bowen, G.D.; Greenhalgh, F.C. (1978) A combined fluorescent antibody and soil sieving technique to count chlamydospores of *Phytophthora cinnamomi* in soil. *Soil Biology and Biochemistry* **10**, 437-438.

Malajczuk, N.; McComb, A.J.; Parker, C.A. (1975) An immunofluorescence technique for detecting *Phytophthora cinnamomi* Rands. *Australian Journal of Botany* **23**, 289-309.

Marshall, M.R.; Partridge, J.E. (1981) Immunochemical identification of *Fusarium moniliforme* ribosomes from diseased corn (*Zea mays* L.) stalk tissue. *Physiological Plant Pathology* **19**, 277-288.

Merz, W.G.; Burrell, R.G.; Gallegly, M.E. (1969) A serological comparison of six heterothallic species of *Phytophthora*. *Phytopathology*. **59**, 367-370.

Miyazaki, T.; Hayashi, O.; Ohshima, Y.; Yadomae, T. (1979) Studies on fungal polysaccharides: the immunological determinant of the serologically active substance from *Absidia cylindrospora, Mucor hiemalis* and *Rhizopus nigricans*. *Journal of General Microbiology* **111**, 417-422.

Mohan, S.B.; Ride, J.P. (1982) An immunoelectrophoretic approach to the identification of progressive and fluctuating isolates of the hop wilt fungus *Verticillium albo-atrum*. *Journal of General Microbiology* **128**, 255-265.

Mohan, S.B.; Ride, J.P. (1983) Fluorescent-antibody staining of conidia of isolates of *Verticillium albo-atrum* in relation to their virulence for hop. *Journal of General Microbiology* **129**, 3581-3589.

Moore, G.E.; Gerner, R.E.; Franklin, H.A. (1967) Culture of normal human leukocytes. *Journal of the American Medical Association* **199**, 519-524.

Morton, D.J.; Dukes, P.D. (1967) Serological differentiation of *Pythium aphanidermatum* from *Phytophthora parasitica* var. *nicotianae* and *Ph. parasitica*. *Nature, London* **213**, 923.

Oi, V.T.; Herzenberg, L.A. (1980) Immunoglobulin-producing hybrid cell lines. In *Selected methods in cellular immunology* (B.G. Mishell; S.M. Shiigi, eds), 351-372. San Francisco; W.H. Freeman.

Pepys, J.; Longbottom, J.L. (1978) Immunological methods in mycology. In *Handbook of experimental immunology* Vol. 3 . *Application of immunological methods* (D.M. Weir, ed),41.1-41.27. Oxford; Blackwell Scientific Publications.

Pontecorvo, G. (1976) Production of indefinitely multiplying mammalian somatic cell hybrids by polyethylene glycol (PEG) treatment. *Somatic Cell Genetics* **1**, 397-400.

Preece, T.F. (1968) The fluorescent antibody technique for the identification of plant pathogenic fungi. In *Chemotaxonomy and Serotaxonomy* (J.G. Hawkes, ed.), 111-114. London; Academic Press.

Preece, T.F. (1971) Immunological techniques in mycology. In *Methods in Microbiology* **4** (C. Booth, ed.), 599-607. London; Academic Press.

Preece, T.F.; Cooper, D.J. (1969) The preparation and use of a fluorescent antibody reagent for *Botrytis cinerea* grown on glass slides. *Transactions of the British Mycological Society* **52**, 99-104.

Rappuoli, R.; Piazzi, S.E.; Severini, R. (1982) Comparison of extracts of *Fusarium graminearum* as antigens and three immunization procedures for the production of anti-fusaria sera. *Annali Sclavo* **24**, 64-75.

Scheffer, R.J.; Elgersma, D.M. (1981) Detection of a phytotoxic glycopeptide produced by *Ophiostoma ulmi* in elm by enzyme-linked immunospecific assay (ELISA). *Physiological Plant Pathology* **18**, 27-32.

Schmidt, E.L.; Bankole, R.O. (1962) Detection of *Aspergillus flavus* in soil by immunofluorescent staining. *Science* **136**, 776-777.

Schmidt, E.L.; Bankole, R.O. (1963) The use of fluorescent antibody with the buried slide technique. In *Soil Organisms* (J. Doeksen; J. van der Drift. eds.), 197-204. Amsterdam; N. Holland Publishing.

Schmidt, E.L.; Bankole, R.O. (1965) Specificity of immunofluorescent staining for study of *Aspergillus flavus* in soil. *Applied Microbiology* **13**, 673-679.

Schmidt, E.L.; Biesbrock, J.A.; Bohlool, B.B.; Marx, D.H. (1974) Study of mycorrhizae by means of fluorescent antibody. *Canadian Journal of Microbiology* **20**, 137-139.

Scott, S.W. (1981a) Contamination of seed of white clover (*Trifolium repens*) with mycelium of a *Sclerotinia* species. *Transactions of the British Mycological Society* **77**, 671-674.

Scott, S.W. (1981b) Serological relationships of three *Sclerotinia* species. *Transactions of the British Mycological Society* **77**, 674-676.

Severini, R.; Rappuoli, R.; Piazzi, S.E.; Tarli, P. (1982) Detection of soluble Fusaria antigens by an immunoenzymatic technique. *Annali Sclavo* **24**, 560-567.

Tarr, S.A.J. (1972) *Principles of Plant Pathology*. London; Macmillan.

Voller, A.; Bidwell, D.E.; Bartlett, A. (1979) *The enzyme-linked Immunosorbent Assay ELISA. A guide with abstracts of microplate applications.* Guernsey; Dynatech Europe.

Warnock, D.W. (1971) Assay of fungal mycelium in grains of barley, including the use of the fluorescent antibody technique for individual fungal species. *Journal of General Microbiology* **67**, 197-205.

Warnock, D.W. (1973) Use of immunofluorescence to detect mycelium of *Alternaria, Aspergillus* and *Penicillium* in barley grains. *Transactions of the British Mycological Society* **61**, 547-552.

Weemen, B.K. van,; Schuurs, A.H.W.M. (1971) Immunoassays using antigen-enzyme conjugates. *Federation of European Biochemical Societies Letters* **15**, 232-236.

White, D.G. (1976) The preparation and use of a fluorescent antibody reagent for the detection of *Pythium graminicola*. *Phytopathology* **66**, 523-525.

Wyatt, P.A.; Di Menna, M.E. (1981) An immunofluorescent counting method for tremorgenic penicillia. *New Zealand Journal of Agricultural Research* **24**, 243-246.

Wyllie, T.D.; Devay, J.E. (1970) Immunological comparison of isolates of *Verticillium albo-atrum* and *V. nigrescens* pathogenic to cotton. *Phytopathology* **60**, 1682-1686.

Yalow, R.S.; Berson, S.A. (1959) Assay of plasma insulin in human subjects by immunological methods. *Nature, London* **184**, 1648-1649.

Zola, H.; Brooks, D. (1982) Techniques for the production and characterization of monoclonal hybridoma antibodies. In *Monoclonal hybridoma antibodies: techniques and applications* (J.G.R. Hurrell, ed.) 1-57, Boca Raton, Florida; CRC Press.

Spoilage and Mycotoxins of Cereals and other Stored Products.
Edited by B. Flannigan

Immunoassay of Mycotoxins

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I. Summary

Immunoassays for quantification of a number of mycotoxins, validated for application to a variety of food matrices, have been described. These assays have several advantages over alternative procedures and should find extensive future application. The procedures involved in the setting up and application of an immunoassay for a mycotoxin are discussed.

II. Introduction

Analysis of mycotoxins, the toxic secondary metabolites of fungi, is important because of their occurrence in a variety of foods (including cereals) destined for human and animal consumption. Such analysis is rendered difficult because:

(a) the highly toxic and carcinogenic nature of some mycotoxins means that even low level contamination, perhaps to the ppt level, is important. Methods must therefore be sensitive;
(b) the presence of many compounds of closely related chemical structure that may be non-toxic means that analysis must be specific;
(c) the non-uniform distribution of mycotoxins in natural products means that appropriate, tested sampling plans must be used in order to ascertain the degree of contamination. Methods of analysis should be able to cope with the large numbers of samples generated in these schemes;
(d) there is often a need for analytical results to be obtained quickly to avoid, for example, delays in processing or distribution. Analytical procedures should reflect this need.

Initially, most mycotoxin analysis was carried out using thin-layer chromatography. More recently, high-performance liquid chromatography (HPLC) has been used. Both procedures, though for the most part sensitive enough, are not sufficiently specific to avoid employment of extensive sample clean-up protocols. Thus the rate of sample analysis can be slow. In addition, HPLC equipment is expensive.

Immunoassays, first described nearly thirty years ago (Yalow & Berson 1959), quickly became established as routine methodology, particularly in clinical chemistry and related areas. The rapid spread of the method was directly attributable to the advantages presented by the new technique. Immunoassays can be extremely specific and sensitive because of the unique high affinity interaction of an antibody for its ligand. Specificity is such that analysis of complex biological matrices can take place with little or no sample pre-purification. The rate of sample analysis is also high because of the

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batchwise nature of the immunoassay operation, which is usually technically simple. Though food analysts have only comparatively recently become more aware of immunological techniques, it is clear that the benefits could be usefully applied to mycotoxin analysis. In this paper, these advantages will be demonstrated, mainly from our own experience.

III. Immunogen synthesis

In order to raise antibodies to mycotoxins, they need to be coupled to a protein or similar biopolymer capable of inducing an immune reaction in a particular animal. The animal will then also produce antibodies capable of recognising the toxin. The nature of the link between toxin and protein is critical in determination of antibody specificity since specificity will be directed towards those parts of the toxin structure distal to the point of linkage. Therefore careful thought needs to be employed before conjugation of toxin, in order to ensure that maximum probability is given to antibody generation of desired specificity characteristics. Toxin can be linked to protein directly, as for ochratoxin A (Morgan *et al.* 1983), or by derivatisation of an existing functional group to form an intermediate that can be covalently bound to protein, as for 3-acetyldeoxynivalenol (Kemp *et al.* 1986) or aflatoxin B_1 (Morgan *et al.* 1986a). Alternatively, the toxin can be partially degraded prior to coupling (Jackman 1985) in order to obtain a broad specificity response.

IV. Production of anti-mycotoxin antisera

Once an immunogen has been synthesised, it can be used to raise antisera in the animal of choice. We prefer to work with rabbits - others have different preferences, such as sheep or guinea-pigs. Administration of immunogen is by standard immunological procedures. After periodic booster injections the blood of the animal is tested for antibody presence and quality. This product reflects the activity of a large number of different antibodies against the toxin, each arising from different cell-lines, and is known as a polyclonal serum product. It is now possible to produce a monoclonal antibody (i.e. a pure antibody preparation consisting of identical molecules) following the work of Kohler & Milstein (1975). Though a monoclonal antibody need not be as specific or of as high affinity as a polyclonal preparation against a mycotoxin, the potential unlimited availability of the monoclonal antibody is attractive in certain circumstances. Monoclonal antibodies against mycotoxins have been produced in our own laboratories (unpublished data) and others (Woychik *et al.* 1984, Candlish *et al.* 1985).

Whether monoclonal or polyclonal antibody preparations are utilised, they should be of the desired quality. The antibody is the key reagent in an immunoassay, and it is difficult to overcome shortfalls in quality by manipulation of assay conditions. Antibody should be available in sufficiently large amounts to allow long-term use and, if possible, dissemination to other laboratories. Specificity should be suitable for the intended purpose of the assay, which may be quantification of several toxins together or just one toxin. Antibody affinity for toxin(s) should be as high as possible so the assay can be rapid and sensitive. Maximum sensitivity is important because it can be traded for even simpler sample preparation procedures. Rather than work with antibodies that do not meet our desired characteristics, we search for appropriate preparations until we find them - a policy that succeeds in the long-term.

V. Setting up an immunoassay

Having obtained antibody, it is used to set up an immunoassay. There are many types of format available, but for mycotoxin analysis it appears that at present the microtitration plate enzyme-linked immunosorbent assay (ELISA) possesses the greatest number of advantages (Morgan, 1985). A microtitration plate consists of 96 wells in moulded polystyrene. One of the assay components, anti-mycotoxin antibody or a standard amount of a toxin-protein conjugate, is immobilised to the wall of each well. Successive incubations, depending on whether the assay is of the direct or indirect type, take place and culminate in addition of substrate and development of colour. The amount of colour is dependent on the concentration of toxin initially present in the analyte.

We have preferred to work on indirect methods of assay (Morgan 1985), the stages of which are as follows:

(i) Immobilise hapten to the plate. If stored dry, "coated" plates are stable for at least a year.
(ii) Add sample extract, or standard quantity of toxin to appropriate wells.
(iii) Add anti-toxin antibody to all wells. Incubate. Antibody will become distributed between immobilised toxin and toxin in solution according to how much of the latter is present. Remove non-immobilised material by washing each well several times.
(iv) Add enzyme-labelled antibody active against the anti-toxin antibody. Such products are commercially available. Incubate. The second antibody will become bound to immobilised anti-toxin antibody. Again, remove unbound material by washing.

(v) Add substrate. Colour will develop at a rate dependent on the amount of enzyme present - which will be related to how much analyte was present in the initial sample or standard. Quantification of unknowns is by reference to standard curves.

I. Application of mycotoxin immunoassays

Though there are many reports describing mycotoxin immunoassays in the literature, few have been used outside the laboratory of inception. The reasons for this are two-fold: either the antiserum has not been of appropriate quality or it has not been used for a correctly validated application. Standard curves for toxins in buffer obtained by immunoassay are meaningless without a validated application. In addition, each new application and each change in sample preparation or assay procedure must be re-validated.

Validation of an immunoassay requires a study of the effects in the assay of negative sample extracts; a study of the behaviour of spiked sample extracts; and looking at known naturally contaminated extracts (including response linearity). Potential interferences are removed by changing or extending the sample extraction procedure. Wider acceptance of an immunoassay in the analytical community will be gained only by seeing the assay in action and its performance against conventional (validated!) procedures.

We have validated assays for aflatoxin B_1 determination in peanut butter (Morgan *et al.* 1986a), ochratoxin A and sterigmatocystin determination in barley (Morgan *et al.* 1983, Morgan *et al.* 1986b) and 3-acetyldeoxynivalenol determination in rice (Kemp *et al.* 1986). These assays have low limits of detection (usually between 1 and 10 pg toxin) which, together with assay specificity, are exploited by devising simple and rapid sample extraction procedures. Often this takes the form of toxin solubilisation from the solid matrix, followed directly by ELISA. As well as being much quicker than conventional sample clean-up, there are beneficial effects on assay cost.

A further development in mycotoxin immunoassays is the commercial availability of assay kits. These are starting to appear in the UK and USA, and will make available to a wider audience all the benefits of the immunological technique. It is envisaged that in the years ahead, immunoassay will be the analytical method of choice for mycotoxin determination in cereals and other commodities.

References

Candlish, A.A.G.; Stimson, W.H.; Smith, J.E. (1985) A monoclonal antibody to aflatoxin B_1: detection of the mycotoxin by enzyme immunoassay. *Letters in Applied Microbiology* **1**, 57-61.

Jackman, R. (1985) Determination of aflatoxins by ELISA with special reference to M_1 in raw milk. *Journal of the Science of Food and Agriculture* **36**, 685-698

Kemp, H.A.; Mills, E.N.C.; Morgan, M.R.A. (1986) Enzyme-linked immunosorbent assay of 3-acetyldeoxynivalenol applied to rice. *Journal of the Science of Food and Agriculture* (in press).

Kohler, G.; Milstein, C. (1975) Continuous cultures of fused cells producing antibody of predefined specificity. *Nature (London)* **256**, 495-497.

Morgan, M.R.A.; McNerney, R.; Chan, H.W.-S. (1983) Enzyme-linked immunosorbent assay of ochratoxin A in barley. *Journal of the Association of Official Analytical Chemists* **66**, 1481-1484.

Morgan, M.R.A. (1985) Newer techniques in food analysis-immunoassays and their application to small molecules. *Journal of the Association of Public Analysts* **23**, 59-63.

Morgan, M.R.A.; Kang, A.S.; Chan, H.W.-S. (1986a) Aflatoxin determination in peanut butter by enzyme-linked immunosorbent assay. *Journal of the Science of Food and Agriculture* (in press).

Morgan, M.R.A.; Kang, A.S.; Chan, H.W.-S. (1986b) Production of antisera against sterigmatocystin hemiacetal and its potential for use in an enzyme-linked immunosorbent assay for sterigmatocystin in barley. *Journal of the Science of Food and Agriculture* (in press).

Woychik, N.A.; Hinsdill, R.D.; Chu, F.S. (1984) Production and characterisation of monoclonal antibodies against aflatoxin M_1. *Applied and Environmental Microbiology* **48**, 1096-1099.

Yalow, R.S.; Berson, S.A. (1959) Assay of plasma insulin in human subjects by immunological methods. *Nature (London)* **184**, 1648-1649.

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Cytotoxicity testing for mycotoxins

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I. Summary

Cell culture methods have been adopted to assay toxicity in animal feedingstuffs. All uncontaminated straight feeds give negative results while the majority of compounded feeds are usually positive, due, in some cases, to the presence of certain additives. The method has to accommodate this finding if it is to be useful as a screen of feed toxicity to animals. A basal level of cell toxicity has been established for compounded feeds which permits this.

II. Introduction

Mycotoxins have been known for many years to cause problems in animal health. In order to establish whether or not the problem is due to mycotoxins the outbreak of disease must fit the following criteria:

1. The problem must be feed associated.
2. No significant micro-organisms have been isolated.
3. The disease cannot be transferred.
4. The disease is not contagious.
5. The disease is not infectious.
6. When the suspect feed is withdrawn an improvement should be seen in the health of the animals.

Although a health problem may appear to be feed-related, the symptoms shown by the animals may not relate to the classical syndromes demonstrated in laboratory animals in mycotoxin trials. Farm animals are exposed to a group or mixture of toxins while laboratory animals are fed one pure toxin. Also, environmental conditions on the farm are extremely variable, as are the physical conditions of the animals, and their food intake is often difficult to assess. Nearest to the laboratory situation are poultry; the conditions of housing and temperature are controlled and the nutritional value and intake of food is monitored along with performance. Pigs are the next most well-monitored animals and, like poultry, are fed basically a cereal diet. Cattle, sheep and deer have the most varied conditions; some are housed, others are kept outside. The food intake of ruminants is almost impossible to determine. The diet consists of cereals and compounded feed plus forage, i.e. silage, hay, haylage or straw; turnips or potatoes may also be fed. The effects that mycotoxins have on animals differ from species to species, and within species, depending on condition of the animal and the sex (Table 1).

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Table 1. *Acute toxicity of aflatoxin B_1*

Species	LD_{50} single dose (mg/kg body weight)	Species	LD_{50} single dose (mg/kg body weight)
Duckling	0.3	Chicken	6.0–16.0
Rainbow trout	0.5–1.0	Rat (male)	7.0
Pig	0.6	Mouse	9.0
Sheep	1.0–2.0	Rat (female)	18.0
Baboon	2.0		

We have, therefore, a very complex situation when suspected mycotoxicoses occur. The problem is not always clarified when the feedingstuffs are examined for mycotoxins. These compounds are usually detected in animal feedingstuffs by chemical analysis, using a solvent extraction method followed by a cleanup process and visualisation of the mycotoxins by thin layer, gas liquid or high pressure liquid chromatography. The number of mycotoxins which can be identified in this manner depends on the method used. For example, there are several techniques whereby up to at least 22 mycotoxins can be identified (Nawotny *et al.* 1982). A review of multi-mycotoxin methods was carried out by Steyn (1981). The drawback with all these tests is that pure mycotoxin standards are required for all the mycotoxins examined. At least 800 toxic metabolites of the fungi have been identified, making the feasibility of testing for all known mycotoxins impossible. In recent years, immunoassay techniques have been developed to identify certain mycotoxins in blood and other materials. The method which may become most useful is the enzyme-linked immunosorbent assay (ELISA). Antibody containing the toxin to be assayed is required for this test. Immunoassay techniques have the same shortcomings for the person investigating the field problem as chemical analysis, that is they only show the presence of toxins tested for, or those for which antigens of mycotoxins or pure chemical mycotoxins are available. For example, a disease syndrome seen in animals maybe due to PR-toxin or tryptoquivalone, and if these compounds are not included in the visualisation of the chemical method a negative result will be recorded.

The approach adopted at ESCA is to use cell cultures to detect toxicity in conjunction with chemical analyses. This technique was first used by Robb *et al.* (1982) when investigating a feed-related problem in broiler chickens. Human epithelial cells (HEp2) were used and by this means, plus chemical analysis, various members of the trichothecene group of compounds were found in the cereal components of the feed. HEp2 cells are particularly sensitive to the trichothecenes. Since then, work had been carried out to develop tissue culture methods as a routine screen. Different cell lines, for example HEp2, Chang liver, Chinese hamster ovary (CHO), Vero, McCoy and baby hamster kidney (BHK-21) have been tried in an attempt to detect organ-specific toxins. The feedingstuffs examined are made up of routine samples, "query safe to feed" or samples associated with disease problems. The samples are split and examined as shown in Fig. 1.

III. Cytotoxicity test

The cells are maintained in suitable media until confluent and then transferred by trypsinization into fresh media and pipetted into microtiter plates, 0.25 ml per well (2×10^4 cells). After 24 hours at 37°C, when the cells are confluent, 1 μl of extract from the feedingstuff to be analysed is added to a well. Each feedingstuff extract is assayed in triplicate. After 24 hours' incubation at 37°C the medium is decanted, and the cells fixed with ethanol before staining with Giemsa's stain (Gurr's improved R66, BDH). They are then examined microscopically and scored 0-4: 0, no effect; 1, 25% of cells affected; 2, 50% cells affected; 3, 75% cells affected; 4, 100% cells affected.

IV. Toxicity of feedingstuffs

Results of these investigations have shown that straight feeds, such as cereals, beans and forage, considered to be relatively uncontaminated (10^4 fungi/g or less), are non-toxic at the concentrations of extracts tested (Table 2). Some mycotoxins, when present in feeds, are not cytotoxic, e.g. deoxynivalenol in wheat - this may be present at too low a concentration to be toxic. The same toxin in barley (Table 2) may be present at a higher concentration (which could be tested by diluting extract) or there may be another, chemically undetected toxin present. Ochratoxin is predominantly detected in extract II and is more specific to Chang liver cells (Table 2).

In the case of highly contaminated or uncontaminated straight feeds with no chemically visualised toxin, there may still be toxicity which, in the case of uncontaminated feed, would be entirely

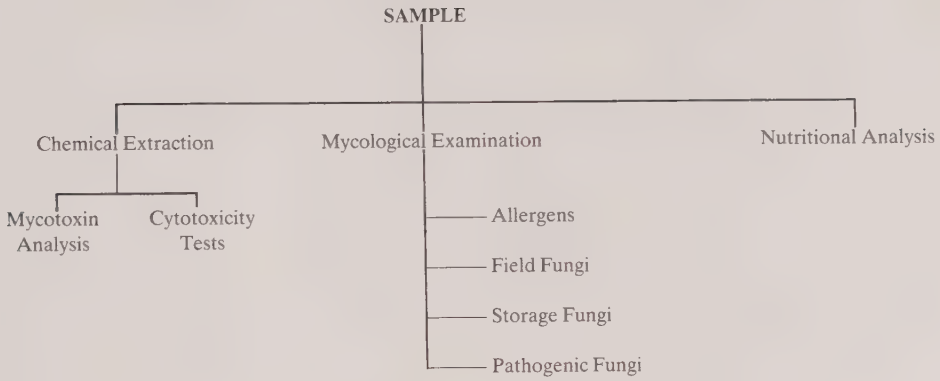

Fig. 1. Types of examination carried out on feed stuff samples

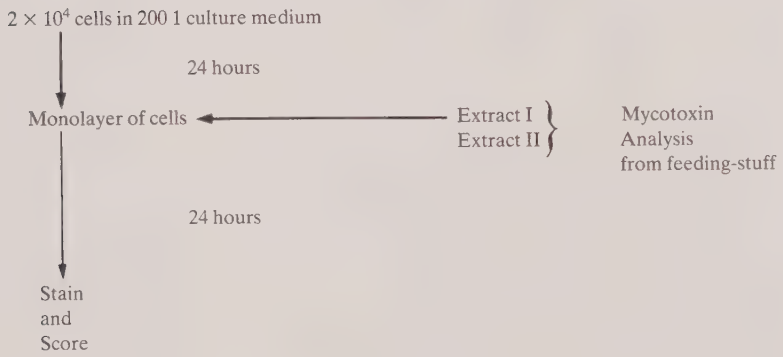

Fig. 2. The Cytoxicity Test

unpredicted. In these cases, the feeds would be shown to be unsafe, perhaps owing to an undetected mycotoxin. No growth regulators, desiccants or fungicides were toxic to the cells at the levels retained on the crops (Table 3).

Compounded feeds, often comprising straight feeds, fats, additives and minerals as pellets, meal or cobs, were consistently cytotoxic (Table 4). Maximum toxicity was recorded in a number of cases and extract I repeatedly gave a score of at least two in HEp2 cells. In the case of beef pellets (*), the constituent straight feeds were available for testing and although these were toxic they had a different mycological population and no deoxynivalenol was detected. Fungi in the straight feeds may be killed if heat is used during the production of compounded feeds, although mycotoxins may not be destroyed. Spoilage of compounded feeds may occur during storage.

Feeds containing known additives were toxic, but Table 5 shows that only Romensin, at the concentrations tested, is toxic to HEp2 cells. The disease-associated pig meal (Table 4) has relatively low fungal contamination and no chemically detectable mycotoxin, but extracts I and II are toxic to several cell lines.

As a result of these studies in compounded feeds, a basal toxicity score of 2, in HEp2 cells, was established for extract I. Levels higher than this were considered to be significant.

Table 2. *The cytotoxicity of extracts I and II of contaminated and non-contaminated feedstuff*

Feed	Fungi/g	Toxin	Cell lines: HEp2 Ex I	HEp2 II	McCoy Ex I	McCoy II	BHK-21 Ex I	BHK-21 II	Chang Ex I	Chang II
Wheat	10^4 *Verticillium* 10^2 Yeast	–	0	0	0	0	0	0	0	0
Barley	10^3 *Fusarium* 10^3 *Penicillium*	–	0	0	–	–	0	0	–	–
Wheat	10^4 *Fusarium* 10^6 Yeast	DON	0	0	0	0	0	0	–	–
Barley	10^4 *Fusarium* 10^2 *Absidia*	DON	2½	0	1	0	0	0	–	–
Barley	10^6 *Penicillium* 10^6 *Acremonium* 10^6 *Absidia*	OCH	½	0	1	0	0	0	0	2
Barley	10^5 *Penicillium* 10^5 *Epicoccum* 10^5 *Cladosporium* 10^5 *Aureobasidium*	–	4	2	2½	0	–	–	–	–
Wheat	10^2 *A. fumigatus* 10^4 Yeast	–	4	2	3	1	–	–	–	–

DON, Deoxynivalenol; OCH, Ochratoxin; *A., Aspergillus*
Scale of Cytotoxicity: 0 (no cell death) to 4 (complete cell death); –, not tested

Table 3. *Fungicides, growth regulators and desiccants tested against cells*

Active Agent	Function	Application
cypermethrin	insecticide	250 ml/hec.
carbendicin	fungicide	500 ml/hec.
triodemefon	fungicide	500 ml/hec.
charothalinol	fungicide	2 litre/hec.
tridemorph	fungicide	700 ml/hec.
ethephon	growth regulator	1.75 litre/hec.
chlormequat	growth regulator	1.75 litre/hec.
carbendazim + flutriafol	fungicide	1.25 litre/hec.
paraquat	desiccant	3 litre/hec.
fumicide	fungicide	
fempropimorph	fungicide	1 litre/hec.
diquat	desiccant	3 litre/hec.
glyphosphate	desiccant	1–4 litre/hec.
iprodione	fungicide	2 litre/hec.
prochloraz + carben	fungicide	1.5 litre/hec.
mepiquat chloride + ethephon	growth regulator	1.75 litre/hec.
propiconazole	fungicide	500 ml/hec.

V. Conclusion

Tissue culture methods are useful indicators of toxicity, particularly for straight feeds. Compounded feeds are generally more toxic to cells but, despite higher basal toxicity levels, it was considered worthwhile to analyse compound feeds with this method. For both classes of feedstuffs, the assay proves most useful when mycological and chemical indicators give negative results.

Table 4. *The cytotoxicity of extracts I and II of compounded feeds*

Feed	Fungi/g	Toxin	Cell lines HEp2 Ex I	HEp2 II	McCoy Ex I	McCoy II	BHK-21 Ex I	BHK-21 II
Barley	10^5 *Penicillium* 10^5 *Cladosporium* 10^5 *Epicoccum* 10^5 *Aureobasidium*	–	4	2	2½	0	–	–
Wheat	10^6 Yeast 10^5 *A. fumigatus* 10^5 *A. repens*	–	3	1½	0	0	–	–
Beef Pellets	10^4 *A. fumigatus* 10^4 *Absidia*	DON	4	0	1½	½	–	–
Beef pellets	10^7 *A. fumigatus* 10^7 *Verticillum*	DON	3	½	2	2	–	–
Pellets + Romensin	10^4 *A. glaucus*	–	3	½	3	0	1	½
Pellets + Avotan	10^4 *A. repens* 10^4 *A. candidum*	–	3½	½	3	0	–	–
Pig meal (Disease associated)	10^3 *A. candidum* 10^3 *Streptomyces* 10^2 *A. fumigatus* 10^2 *Absidia*	–	2	2½	3	3½	2½	3

DON, Deoxynivalenol; *A., Aspergillus*
Scale of Cytotoxicity: 0 (no cell death) to 4 (complete cell death); –, not tested

Table 5. *The cytotoxicity of additives*

Additive	Cell Line	Volume added to wells 1µl	5µl	10µl	20µl
Romensin	HEp2	2½	2½	2	2½
Avotan	HEp2	0	0	0	½

Scale of cytotoxicity: 0 (no cell death) to 4 (complete cell death)

Components of compound feeds, such as the additive Romensin, may be toxic although other substances, e.g. toxins or unknown additives, may also be involved. This occurred in a case of poultry ascites where added recycled animal tallows proved to be a contributory factor in the disease. These feeds recorded scores of 4 in nearly all cell lines.

In vitro cell culture is a useful adjunct to existing mycotoxin detection methods, but could be used on its own to detect toxic feedstuffs.

References

Nawotny, P.; Baltes, W.; Kronert, W.; Weber, R. (1982). Screening method for determination of 22 mycotoxins in mouldy foods. *Proceedings, Fifth IUPAC Symposium on Mycotoxins and Phycotoxins*, 36-39.

Robb, J.; Kirkpatrick, K.; Norval, M. (1982). Association of toxin-producing fungi with disease in broilers. *Veterinary Record* **111**, 389-390.

Steyn, P.S. (1981) Multimycotoxin analysis. *Pure and Applied Chemistry* **53**, 892-902.

oilage and Mycotoxins of Cereals and other Stored Products.
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Microorganisms and Microbiological Rapid Methods in the Bioassay of Mycotoxins

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I. Summary

Many mycotoxins show some antimicrobial activity as well as toxicity to mammals. Indeed, a number of fungal metabolites showing potentially useful antibiotic activity have been rejected by the pharmaceutical industry because of their mammalian toxicity. A few species of yeasts, protozoa, algae and bacteria have shown promise in the development of bioassays for individual mycotoxins, but such assays, usually lacking specificity, are frequently time consuming. In food and medical microbiology there has been a considerable drive in the development of rapid methods for assessing microbial activity. The possibility that these methods may be applied to the bioassay of mycotoxins will be explored, with particular reference to the use of impedance measurements.

II. Introduction

As an antibiotic, the compound now widely known as patulin was rediscovered several times and provided with a name reflecting the source of its discovery: clavacin, claviformin, expansin and clavatin produced by *Aspergillus clavatus, Penicillium claviforme, P. expansum* and *P. patulum* . The review of Ciegler *et al.* (1971) added a number of other synonyms for patulin arising from commercial interest in its wide spectrum antibiotic activity (penicidin, mycoin, tercinin and leucopin). In the event, patulin proved to be too toxic to be useful as an antibiotic and was rediscovered, after a brief period, as the agent responsible for the deaths of cattle fed on mouldy barley malt (Jacquet *et al.* 1963).

Mycotoxins are a diverse range of fungal secondary metabolites which give rise to toxicological phenomena as diverse as nephrotoxicity, hepatotoxicity, carcinogenicity, oestrogenicity, activity against the central nervous system, the sympathetic nervous system and the immunological defence system. With this wide range of biological activity it is not surprising that there are no satisfactory microbiological assays covering all mycotoxins. Nevertheless, it is possible that the careful use of a small range of microbial systems may provide useful information, in conjunction with other biological assays, in a preliminary screen for the presence of mycotoxins in foods, animal feeds and food and feed additives. Watson & Lindsay (1982) have made a critical appraisal of biological methods for the detection of mycotoxins.

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III. Bacteria

The differences between prokaryotes and eukaryotes are sufficiently profound that it is unlikely that bacteria will be useful in a general screen for mycotoxin activity, but a number of mycotoxins have antibacterial activity, and bioassays using bacteria have been developed for them. Hole-in-plate and disc assays are very conveniently carried out on plates of nutrient agar seeded with spores of *Bacillus subtilis*, and such an assay has been developed for patulin (Reiss 1975a). Although aflatoxin has some activity against Gram-positive spore formers such as *Bacillus megaterium, B. brevis* (Burmeister & Hesseltine 1966) and the thermophile *B. stearothermophilus* (Reiss 1975b), a number of significant mycotoxins, such as rubratoxin and the trichothecenes, cannot be assayed using these methods.

IV. Fungi

Many filamentous fungi are sensitive at some stage during their development to a wider range of mycotoxins than are the bacteria. Thus a number of moulds, including the producing species, have been shown to be inhibited by aflatoxin B_1 (Lillehoj *et al.* 1967) and *Aspergillus niger* is inhibited in its growth by as little as 15 μg rubratoxin B/ml (Moss & Badii,1980). The single-celled yeasts could be useful in a routine bioassay based on traditional plate assay methodology if they were sufficiently sensitive. In an alternative approach using the fermentative activity of *Saccharomyces cerevisiae*, which can be measured by the rate of CO_2 production, concentrations of aflatoxin and diacetoxyscirpenol in the range 10 - 100 μg/ml can be assayed (Reiss, 1973). Flannigan *et al.* (1985) have pointed out the significance of the inhibitory activity of *Fusarium* toxins against yeasts to the brewing industry. They showed that the growth of *S. cerevisiae* was inhibited in a dose dependent manner by both zearalenone and the trichothecene, T-2 toxin.

During a survey of 75 yeasts isolated from a variety of sources, Sukroongreung *et al.* (1984) found that the minimum inhibitory concentration (MIC) of T-2 toxin varied from 1.0 to greater than 8.0 μg/ml. They found *Kluyveromyces fragilis* to be the most sensitive and demonstrated that, if a sensitive yeast strain is to be used for the bioassay of trichothecenes, it is important to standardise inoculum levels, temperature and time of incubation before reading the results. In an earlier study, Schappert & Khachatourians (1984) had shown that certain reagents in the medium, which may influence the integrity and functioning of the cell membrane, alter the sensitivity of species of *Saccharomyces* to T-2 toxin. Thus ethanol, cetyltrimethylammonium bromide and Triton X-100 all increase the sensitivity of the yeast to the toxin.

V. Protozoa

The possibility that protozoa may be used for bioassays of mycotoxins is attractive because they would seem to be more closely related to animal cells. However, in general, most laboratories would have difficulties in maintaining cultures for routine use and the observations of toxicological phenomena require intensive microscopic observations. The ciliate, *Tetrahymena pyriformis*, is very amenable to routine laboratory culture and its sensitivity to the rubratoxins has provided the basis of a sensitive bioassay for these compounds (Wyatt & Townsend 1974, Hayes *et al.* 1974).

VI. Rapid methods in microbiology

Traditional methods of obtaining quantitative information about the growth of microorganisms have usually been demanding on time or have required skill and commitment to microscopic observation. Improvements in the reproducibility and sensitivity of automated methods of assessing microbial activity have given rise to considerable interest in their application to medical, industrial and food microbiology (Tilton 1982).

Of particular interest have been those methods for measuring microbial activity rather than number of viable microbial propagules. These include such methods as microcalorimetry, in which the heat produced by actively growing cultures can be measured; radiometry by which the $^{14}CO_2$ liberated from a radiolabelled carbon source can be reliably and quantitatively measured; luminometry as a means of measuring the ATP present in all living and active cells; and impedimetry, by which the changes in the electrical properties of a medium resulting from active growth of a microorganism can be measured.

1. Impedance Microbiology

It has been known for many years that the growth of microorganisms in a medium causes changes in the electrical properties, such as conductivity, of that medium, and that the

measurement of thesechanges provides a measure of microbial activity. Imposition of an electric field on an electrolyte solution, such as a microbiological medium, will result in the movement of ions (conductance, G) and the storage of electrical energy (capacitance, C), both of which contribute to the impedance (Z) of the medium to an alternating current.

$$Z = \sqrt{\left(\frac{1}{G}\right)^2 + \left(\frac{1}{2\Pi fC}\right)^2}$$

Microbial metabolism can cause changes in both the conductance and capacitance of a medium and there have been remarkable advances in instrumentation for reproducibly measuring these changes. The two most widely available instruments are the Bactometer Microbial Monitoring System and the Malthus Microbiological Growth Analyser. A detailed account of impedance microbiology and a description of the development of instrumentation are provided by Eden & Eden (1984).

The Bactometer 32 can be used to study the growth activity in 32 samples simultaneously at a constant temperature and has been used to study the sensitivity of microorganisms to trichothecenes (Adak *et al.* 1986). During a preliminary study involving about 20 strains each of bacteria and moulds, and 8 strains of yeast, it was found that 0.4% yeast extract broth (Oxoid L21) was the medium of choice for the bacteria, whilst 2% malt extract broth (Oxoid L39) supplemented with 2% glucose was best for yeasts and moulds. T-2 toxin, diacetoxyscirpenol, deoxynivalenol, verrucarin A and roridin A were each dissolved in dichloromethane/methanol (95:5) and diluted with the appropriate sterile broth. Each test consists of a reference well and a sample well, the latter differing only in the presence of an inoculum of the test organism. The instrument records the impedance ratio of each of the 32 tests at approximately 1.5 second intervals thus any one test is monitored every 90 seconds.

$$\text{IMPEDENCE RATIO} = \frac{Z_R}{Z_R + Z_S} \quad \begin{cases} Z_R = \text{Impedence of reference} \\ Z_S = \text{Impedence of sample} \end{cases}$$

For defined conditions of inoculum level, medium composition and temperature each strain will produce a characteristic pattern in the rate of change of impedance ratio with time. This pattern is characterised by both a delay in the initial appearance of a growth associated signal (detection time) and the time at which the maximum rate of change occurs (Fig. 1). The presence of an antimicrobial agent influences both these times, thus making it possible to detect antimicrobial activity at concentrations below those at which complete inhibition of growth occurs.

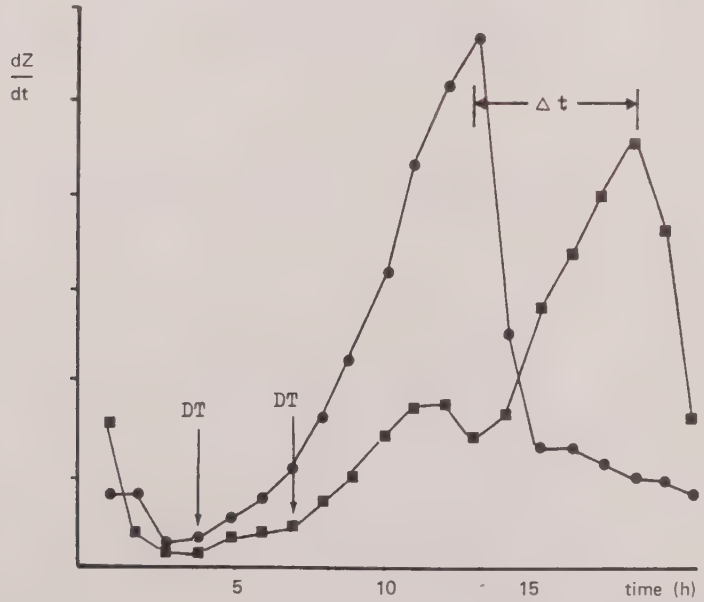

Fig. 1 The effect of T-2 toxin (1.0 μg/ml) on the growth of *Lodderomyces elongisporus* as measured using the Bactometer 32
●——● control; ■——■ + toxin; DT= detection time.

After screening a large number of strains of microorganisms (195 yeasts, 74 moulds and 20 bacteria) for sensitivity to trichothecenes, a strain of the yeast *Hansenula fabianii* was shown to be particularly sensitive. A dose response curve for this organism against T-2 toxin is shown in Fig. 2. from which it can be seen that as little as 0.1 ppm gave a readily discernible response and the detection limit was about 12.0 ppb.

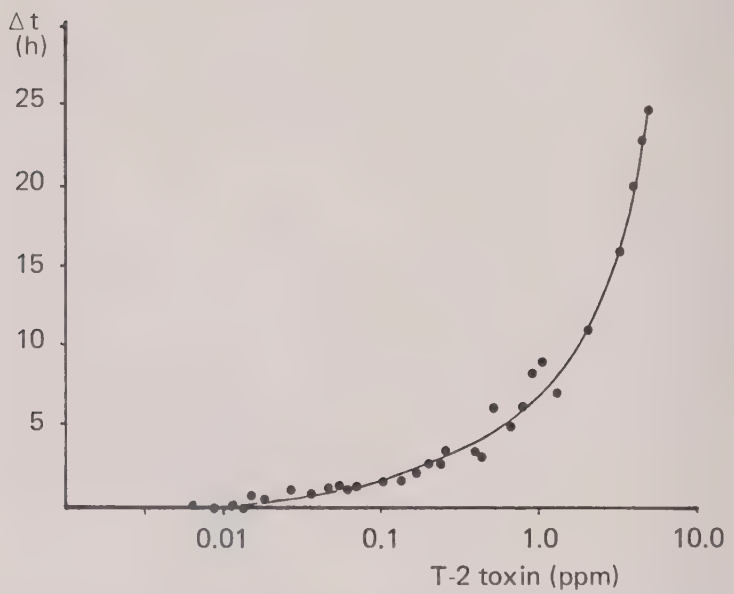

Fig. 2 Dose response curve for T-2 toxin against the yeast *Hansenula fabianii*

Δ t = time lag between the peak of maximum response in the presence and absence of toxin.

At the present, these experiments have only involved the use of pure toxins and it is not known how the responses obtained will be affected by the additional materials present in crude extracts. However, the method has the potential of providing a sensitive assay for trichothecenes, particularly of the T-2 toxin and diacetoxyscirpenol type. Yeasts are less sensitive to deoxynivalenol. Thus, for *Hansenula fabianii* CBS 2521, at concentrations of 1.0 ppm, Δt for T-2 toxin is ca 10 h, for diacetoxyscirpenol 4 h and for deoxynivalenol 0.5 h. The ability of this instrument to handle large numbers of samples might be most important in the routine preliminary screening of foods, feeds and food additives.

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References

Adak, G.K.; Corry, J.E.L.; Moss, M.O. (1986) Use of impedimetry to detect trichothecene mycotoxins. *Journal of General Microbiology* (submitted).

Burmeister, H.R.; Hesseltine, C.W. (1966) Survey of sensitivity of microorganisms to aflatoxin. *Applied Microbiology* **14**, 403-404.

Ciegler, A.; Detroy, R.W.; Lillehoj, E.B. (1971) Patulin, penicillic acid and other carcinogenic lactones, In *Microbial Toxins 6* (A. Ciegler; S. Kadis; S.J. Ajl, eds), 409-434. New York; Academic Press.

Eden, R.; Eden, G. (1984) *Impedance Microbiology*. New York; J. Wiley.

Flannigan,; Morton, J.G.; Naylor, R.J. (1985) *Fusarium* mycotoxins and the malting of barley. In *Trichothecenes and other Mycotoxins* (J. Lacey, ed.), 171-184. Chichester; J. Wiley.

Hayes, A.W.; Melton, R.; Smith, S.J. (1974) Effect of aflatoxin B_1, ochratoxin and rubratoxin on a protozoan, *Tetrahymena pyriformis* HMS. *Bulletin of Environmental Contamination and Toxicology* **11**, 321-325.

Jacquet, J.; Boutibonnes, P.; Cicile, J. P. (1963) Observations sur la toxicité d'*Aspergillus clavatus* pour less animaux. Bulletin de l'Académie Vétérinaire de France **36**, 199-208.

Lillehoj, E.B.; Ciegler, A.; Hall, H.H. (1967) Fungistatic action of aflatoxin B_1. *Experimentia* **23**, 187-188.

Moss, M.O.; Badii, F. (1980) Effect of rubratoxin B on growth, metabolism and morphology of *Aspergillus niger*. *Transactions of the British Mycological Society* **74**, 1-11

Reiss, J. (1973) Influence of the mycotoxins aflatoxin B_1, rubratoxin and diacetoxyscirpenol on the fermentation activity of baker's yeast. *Mycopathologia et Mycologia Applicata* **51**, 337-345.

Reiss, J. (1975a) *Bacillus subtilis*; a sensitive bioassay for patulin. *Bulletin of Environmental Contamination and Toxicology* **13**, 689-691.

iss, J. (1975b) Mycotoxin bioassay, using *Bacillus stearothermophilus*. *Journal of the Association of Official Analytical Chemists* **58**, 624-625.
ıappert, K.T.; Khachatourians, G.G. (1984) Influence of the membrane on T-2 toxin toxicity in *Saccharomyces* spp. *Applied and Environmental Microbiology* **47**, 681-684.
kroongreung, S.; Schappert, K.T.; Khachatourians, G.G. (1984) Survey of sensitivity of twelve genera of yeasts towards T-2 toxin. *Applied and Environmental Microbiology* **48**, 416-419.
ton, R.C. (Ed) (1982) *Rapid Methods and Automation in Microbiology*. Proceedings of the Third International Symposium on Rapid Methods and Automation in Microbiology. Washington; American Society for Microbiology.
atson, D.H.; Lindsay, D.G. (1982) A critical review of biological methods for the detection of fungal toxins in foods and feedstuffs. *Journal of the Science of Food and Agriculture* **33**, 59-67.
yatt, T.D.; Townsend, R.J. (1974) The biossay of rubratoxins A and B using *Tetrahymena pyriformis* strain W. *Journal of General Microbiology* **80**, 85-92.